Suhagcines I and II, Unusual Nucleosides, Diketopiperazines and Further New Secondary Metabolites from Fungal Strains, Terrestrial and Marine Bacteria

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina

zu Braunschweig

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

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1. Referentin:	PD. Dr. Barbara Schulz
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eingereicht am:	18.07.2011
Mündliche Prüfung (Disputation) am:	23.09.2011

Druckjahr 2011

Vorvertöffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakultät für Lebenswissenschaften, vertreten durch die Mentorin der Arbeit, in folgenden Beiträgen vorab veröffentlicht:

Publikationen

Imene Zendah, Naheed Riaz, **Hamdi Abdel Rahim**, Holm Frauendorf, Anja Schüffler, Aly Raies, and Hartmut Laatsch, Chromophenazines from the Terrestrial *Streptomyces* Sp. Ank 315: *J. Nat. Prod.*, submitted Nov. **2010**

Salem Elkahoui, **Hamdi Abdel Rahim**, Olfa Tabbene, Mohamed Shaaban, Ferid Limam and Hartmut Laatsch, *Cyclo*-(His,Leu): A new Microbial Diketopiperazin from a Terrestrial *Bacillus subtilis* strain B38: *J. Nat. Res .*submitted Apr. **2011**

Tagungsbeiträge

Sayed A. Ahmed, **Hamdi Abdel Rahim**, Khaled A. Shaaban, Mahmoud Al Refai, Hafizur Rahman, Heidrun Anke and Hartmut Laatsch: "Butanolides, Acetophenones and Tyrosol Derivatives from *Terrestrial* Streptomyces spp." (Poster) „2nd Göttinger Chemie-Forum". July 3rd 2008. Göttingen, Germany.

Hamdi Abdel Rahim, Hnin Yu Win, Heidrun Anke and Hartmut Laatsch: "Suhagcin I and II Isolated from *Terrestrial* Streptomyces sp." (Poster) „3rd Göttinger Chemie-Forum". July 3rd 2009. Göttingen, Germany.

Imene Zendah, Hnin Yu Win, **Hamdi Abdel Rahim**, Khaled A. Shaabaan and Hartmut Laatsch: "New Phenazine Derivative from *Terrestrial* Streptomyces sp. " (Poster) „3rd. Göttinger Chemie-Forum" July 3rd 2009. Göttingen, Germany.

Dhafer Saber ZINAD, Khaled A. SHAABAN, **Hamdi A. RAHIM**, Muna A. ABDALLA, ، Muhammad BAHI, Heidrun ANKE and Hartmut LAATSCH. "New Isocoumarins from a Terrestrial *Streptomyces* sp." (Poster) „3rd. Göttinger Chemie-Forum" July 3rd 2009. Göttingen, Germany.

I. Zendah, N. Riaz, **H. Abdel Rahim**, A. Raies, H. Laatsch, “A NEW ENDO-PHENAZINE DERIVATIVE FROM A TERRESTRIAL *STREPTOMYCES* SP. ANK315” (Poster) „SSNT-XIX^{èmes} Journées Nationales de Biologie” 05-08 November 2009 Hammamet, Tunisia.

Jana Tiefenau, Frederike Frese, **Hamdi Abdel Rahim**, Hongpeng Wang, Hartmut Laatsch, Michael Steinert: “Specific Inhibition of *Legionella* by Compounds Isolated from *Bacillus licheniformis* and *Bacillus pumilus*” (Poster). 21.11- 24.11.2010 on the 6th. Joint Ph.D. Students Meeting “New trends in infections disease research” in Haus-Schöneberg/Ellwangen, Germany.

Hamdi Abdel Rahim, Mohamed Shaaban, Mohammad Magdy El-Metwally and Hartmut Laatsch: “A new Diketopiperazine Alkaloid from *Aspergillus oryzae*” (Poster) “4th. Göttinger Chemie-Forum” July 8th 2011. Göttingen, Germany.

Acknowledgements

This work was done under the supervision of Prof. Dr. H. Laatsch, Institute of Organic and Bio-molecular Chemistry, University of Göttingen, Germany. I wish to express my sincere gratitude to him for supervising this study. I am deeply appreciative of his sincere support, valuable and constructive discussions, guiding and fruitful advice, which enriched the quality of this work. I am very lucky to be one of his students. I am grateful for the provision of both excellent scientific and working facilities.

I am thankful to PD. Dr. Barbara Schulz for accepting to read this work.

I am deeply thankful to my dear colleagues and employees in Prof. Laatsch's group for their friendship, the warm atmosphere, support, and collaboration during my study and the good times we had together. I am also thankful to all the students associated with me; they have been obedient, and extremely responsive.

A special thanks goes to Dr. P. Facy and M. Al-Refa'i for accepting to read and edit my thesis. I am also indebted to Dipl.-Geol. Mrs. F. Lissy for the microbiological work and some administrative help, Mrs. P. Lappe for administrative help and A. Kohl for technical assistance.

I will not forget my parents, brothers and other family members for all their support, financially and spiritually. I am forever grateful to my mother and late father. Words are inadequate to express my feelings for their financial and moral support. Providing me the opportunity to enrich my academic career was the greatest gift they could have given me. Their love, prayers and encouragement, helped me in every stage of my life. Your eyes have spoken to me!

The credit for this extensive and detailed work in such a wide topic goes to the many hours each day and night that have extended to me to do the task that I may have never been able to do in five years of my life. For this I am grateful to Allah for giving me strength to endure. I am especially grateful to my wife Mrs. Walaa Hassan and my daughter Hala Hamdi Mohamed Desoky for their continuous support, encouragement, love, and understanding during this work.

Hamdi Abdel Rahim

Table of Content

1 Introduction

1.1 Short History of Natural Products

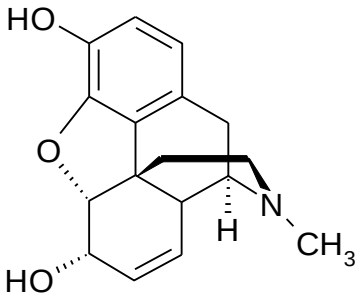
What are the natural products? When did they arise? What is their importance?

Natural products are defined as chemical substances produced by nature; they are not artificial or manufactured. These compounds are produced by microbes, plants, insects or other animals.[1] Nature produces compounds belonging to many chemical groups, such as terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases and so on. Some of them like carbohydrates or proteins are essential for sustaining the life functions like normal growth, development, and reproduction. These chemical compounds are called primary metabolites, while another class with more limited distribution in nature is not necessarily produced under all conditions and is called secondary metabolites. In the vast majority of cases the function of these latter compounds and their benefit to the producing organism is not yet known.[2]

More than three thousand years ago, the ancient Egyptians had used several natural products such as opium, castor oil and rotten bread to treat infections, and during the first century the Roman physician Dioscorides investigated hundreds of plants and wrote the first systematic medical compilation, his book *materia medica*. Further pioneers in using natural products were the Chinese people who applied herbs to treat diseases. In Ayurveda (1500 – 1200 BC), the term used for the traditional medicinal system in India, Sri Lanka and other Asian countries, we find prescriptions of herbal medicines against aging. Later, the Greek physician Galen (129 – 200) described the appearance, properties and use of many plants in his time.[3]

Back in the 19th century, the first active compounds isolated from traditional plants were strychnine, atropine, and colchicine**.** Morphine (**1**), isolated from opium, the processed juice of poppy (*Papaver somniferum*), was produced and commercialised by E. Merck for the first time in 1826.[4] It has been developed as the first drug with a guaranteed purity and is still in clinical use.[5]

In 1929, the pharmacologist Alexander Fleming isolated penicillin (**2**) from *Penicillium notatum*, which was introduced as the first reliable antibiotic in the forties of the last century by Howard Florey and Ernest Chain.[6]



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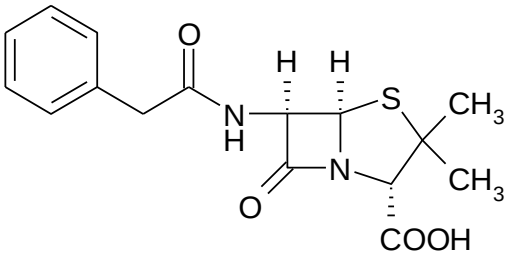

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Until early of 1970, many new antibiotics were isolated from microbes, especially from actinomycetes and fungi. Many of them were applied commercially, and their chemical scaffolds were later used as leads to generate next generations of clinically useful antibiotics by chemical modification.[7]

Secondary metabolites isolated from living microorganisms include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, antitumor agents and growth promoters of animals and plants, and can play great role in medicine, industry and/or agriculture (economics of our society).[8]

Original metabolite	Commercial products[a]	Producing organism
Penicillins	Penicillin G, V, Ampicillin, Methicillin, Amoxicillin, Carbenicillin	*Penicillium* spp., *Aspergillus* spp.
Cephalosporins	MEFOXIN (Cefoxitin), CECLOR (Cefaclor), CLAFORAN (Cefotaxime), ROCEPHIN (Ceftriaxone), CEFTIN (Cefuroxime)	*Acremonium* spp., *Emericellopsis* spp., *Amycolatopsis lactamdurans*, *Streptomyces clavuligerus*
Thienamycin	PRIMAXIN (Imipenem), INVANZ (Ertapenem)	*Streptomyces cattleya*
Erythromycin	ERYTHROCIN, ZITHROMAX (Azithromycin), BIAXIN (Clarithromycin), KETEK (Telithromycin)	*Saccharopolyspora erythraea*
Vancomycin	VANCOCIN	*Streptomyces orientalis*
Fosfomycin	MONURIL	*Streptomyces fradiae*
Mupirocin (pseudomonic acid)	BACTROBAN	*Pseudomonas fluorescens*
Fusidic acid	FUSIDIN LEO[b]	*Fusidium griseum*
Streptogramins	SYNERCID (Dalfopristin/quinupristin)	*Streptomyces pristinaespiralis*
Daptomycin	CUBICIN	*Streptomyces roseosporus*

[a] Trade names are in capitals. Non-capitalized names between parentheses refer to marketed semisynthetic derivatives from the original natural compound. Only some representatives are indicated.
[b] In Canada.

Table 1: Examples of marketed antibiotics originated in microbial natural products [7]

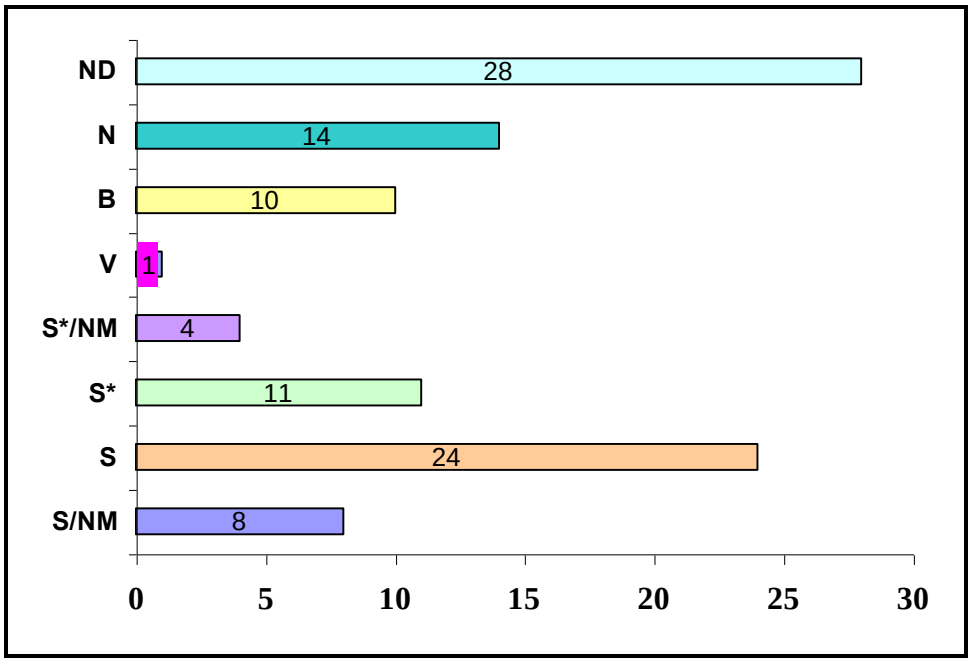


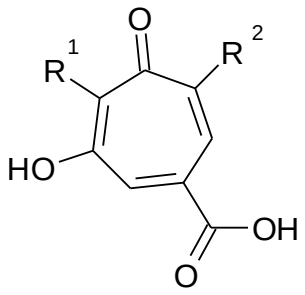
Figure 1: Available anticancer drugs in 1940-2006 by major category sources [9]

"B" Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host, "N" Natural product, "ND" Derived from a natural product and is usually a semisynthetic modification, "S" Totally synthetic drug, often found by random screening/ modification of an existing agent, "S*" Made by total synthesis, but the pharmacophore is/was from a natural product, "V" Vaccine, "NM" Natural product mimic.

1.2 Recently Discovered Metabolites

Actinomycetes and fungi are traditionally the most prolific group in antibiotic production, and have been the focus of most of the efforts by industrial and academic laboratories for the last 60 years to produce a good number of marketed antibiotics [10]. It was proved in 1982 – 2002 when 70 out of the 90 antibiotics on the market were from natural sources.[11] Antibiotics and natural products are intimately linked terms, so that the word "antibiotic" is defined as "a secondary metabolite, produced by microorganisms, which has the ability to inhibit the growth of or even to destroy bacteria and other microorganisms, in a very low concentration".[12] Today nature supplies more than half of the drugs, which are used in many therapeutic categories.[13] Malaria for example caused 863.000 deaths in 2008;[14] it is initiated by dan-

gerous parasitic *Plasmodium* species, which are transmitted through the bite of the mosquito *Anopheles*. Since it is almost impossible to eliminate the vector of transmission,[15] the search for novel and safe antimalarial agents, perhaps with a new mode of action is urgently required in the pharmaceutical industry. Recently a high antimalarial activity was found for puberulic acid (**3a**) [16,17] and stipitatic acid (**3b**), [18] or in the related viticolins A, B (**3c-d**) and C (**4**) isolated from *Penicillium* sp. FKI-4410.[19] The presently most important antimalarial agent is arteether (**5**), a recently developed derivative of artemisinin, isolated from *Artemisia annua;* [20] it has a longer biological half-life time and a higher stability than other artemisinin derivatives.



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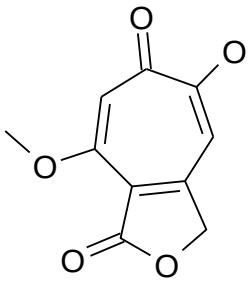

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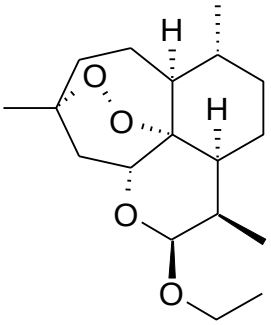
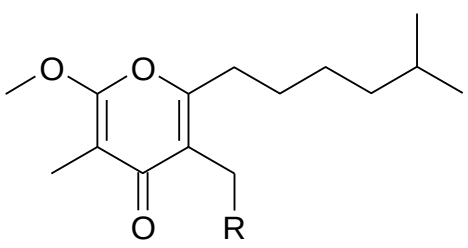
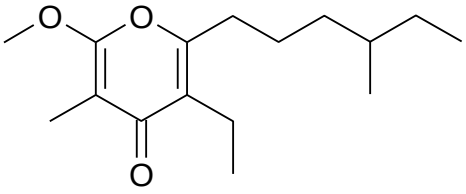


5

	R^1	R^2
(**3a**) Puberulic acid	OH	OH
(**3b**) Stipitatic acid	H	OH
(**3c**) Viticolin A	OCH_3	OH
(**3d**) Viticolin B	OCH_3	OCH_3

Marine actinomycetes are an important source for novel secondary metabolites with a great potential in drug exploration.[21,22] Recently, an isolate of *Marinactinospora thermotolerans* SCSIO 00606 was found to produce three γ-pyrones (**6a-c**) with high cytotoxicity against human cancer cell lines by inhibition of DNA topoisomerase II.[23]

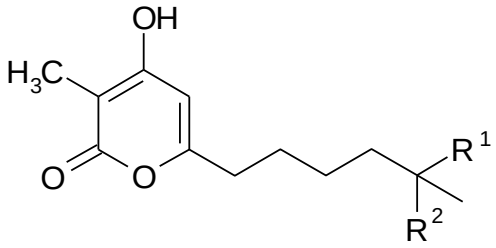




6a: R = H
6b: R = OH

6c

The marine derived *Streptomyces* sp. B 8042 delivered in our group the new pyrones **7a-d** with moderate antibacterial activity: **7b** and **c** showed IC_{50} values of 1.41 µg/ml against different tumour cell lines.[24]



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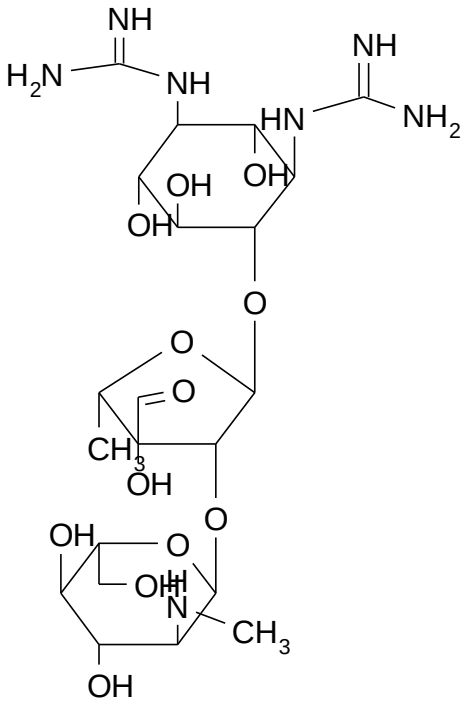
	R^1	R^2
7a	CH_3	OH
7b	CH_3	H
7c	CH_2CH_3	H
7d	$CH(OH)CH_3$	H

Members of the genus *Streptomyces* are well known for their ability to produce secondary metabolites, which play a great role in medicine, industry and/or agricultural.[25] Streptomycetes produce over two thirds of all clinical antibiotics.[26] The history of their discoveries dates back to streptomycin (**8**), soon followed by a huge number of further metabolites isolated from rare actinomycetes. Amongst the clinically useful antibiotics, about 80% were isolated in the period between 1955 to 1962,[27] and in the mid eighties all important groups of antibiotics were discovered: Erythromycin (**9**), amphotericin (**10**) and the antiviral aciclovir and further groups are still playing an important role in our society.[28]



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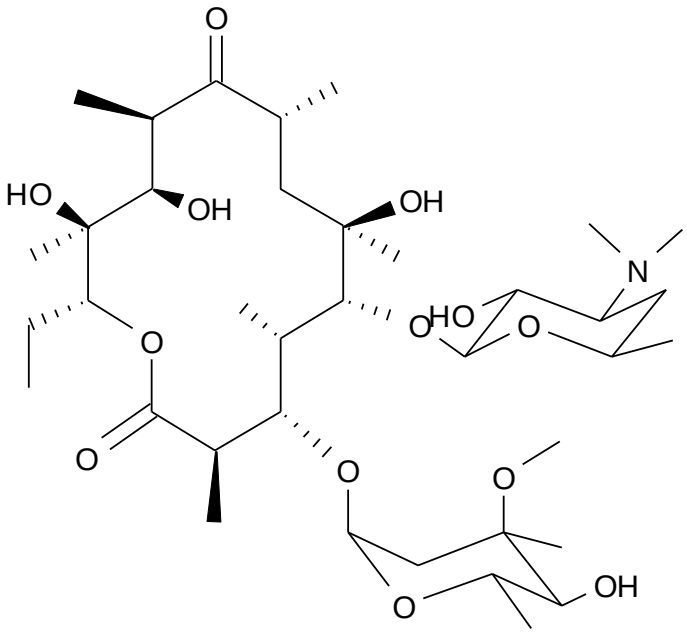

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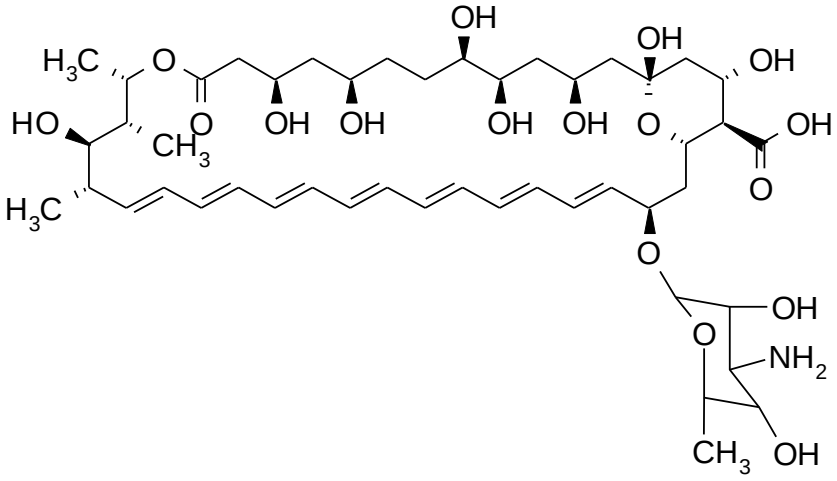
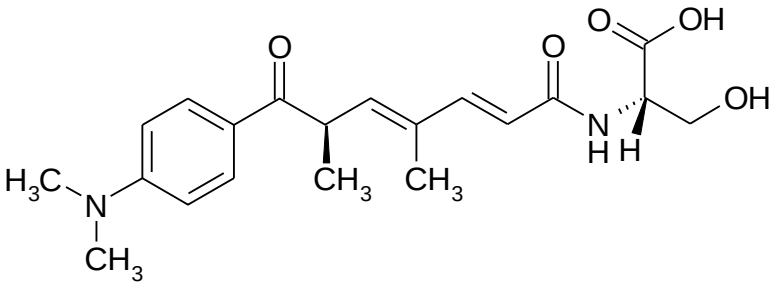


10

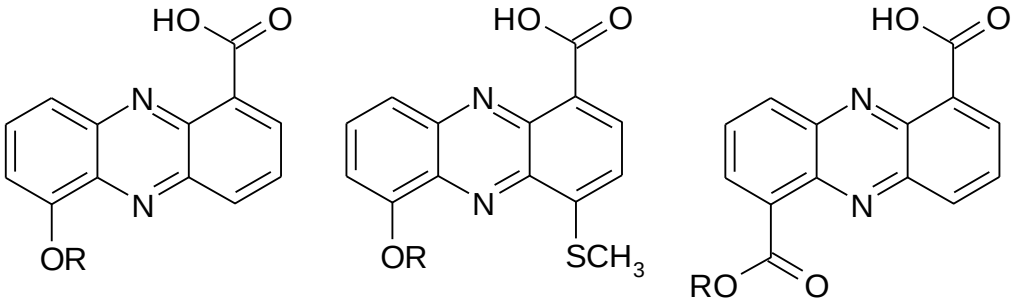
Histone deacetylases (HDACs) are important targets for anticancer drugs. The inhibitors of HDACs can induce cell cycle arrest, promote differentiation and stimulate tumor cell death.[29,30] Recently, the trichostatin analogue JBIR-17 (**11**) was isolated from *Streptomyces* sp. 26634. It is a compound found to be an inhibitor of the cytoplasmic HDAC6, an enzyme that regulates many biological processes.[31]



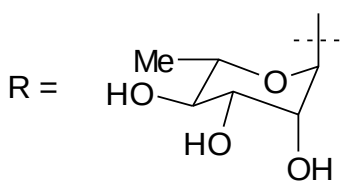
11

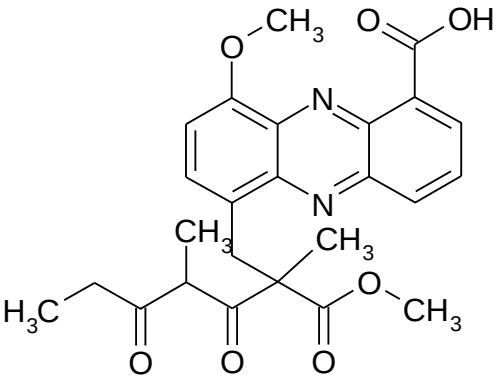
1.3 Antibacterial Compounds from Natural Sources

The chemistry of natural products is principally related to biosynthesis, isolation and structure elucidation of new bioactive secondary metabolites from nature. Phenazines constitute one of the major groups produced by streptomycetes; they display a broad range of activities, such as antibacterial, antimalarial, antitumor, and antiparasitic properties.[32] Recently three new phenazines designated as izumiphenazines (**12a-c**)[33] were isolated from *Streptomyces* sp. IFM 11204. Compounds **12b, c** exhibited synergistic activity against cancer cells according to Jin *et al*.[34] The crude extract of *Streptomyces* sp. ICBB8198 exhibited activity against many kinds of microorganisms, for example *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The work-up of this strain delivered two new phenazines **13, 14**; only the first one showed antimicrobial activity.[35]



12a **12b** **12c**





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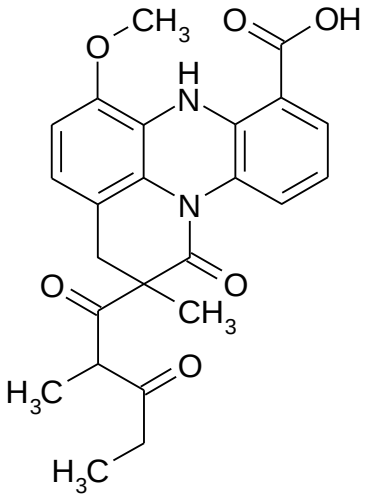
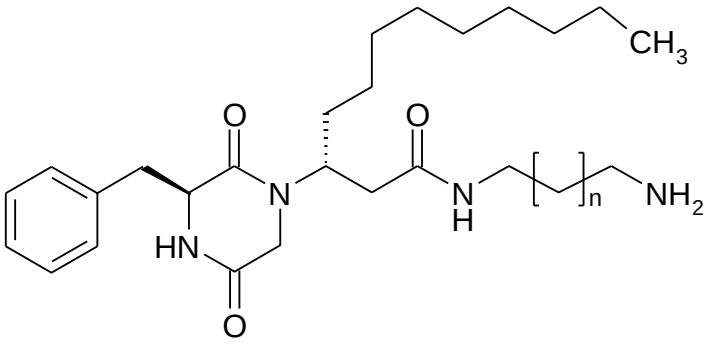


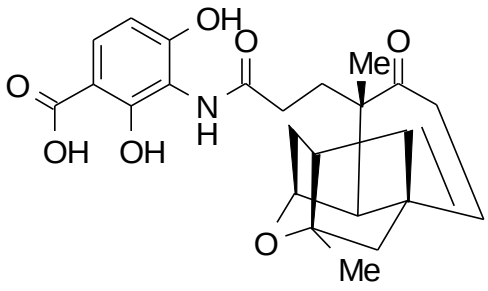
14

Condensation of two amino acid precursors is involved in the biosynthesis of diketopiperazines.[36] Diketopiperazines are well known secondary metabolites produced by plants, bacteria and fungi.[37] The modified diketopiperazine rodriguesines A (**15a,b**)[38] had been isolated from an ascidian of the genus *Didemnum* as a mixture that displayed moderate antibiotic activity against clinical isolates of *Streptococcus mutans* and *Staphylococcus aureus* ATCC6538.



15a: n = 1, **15b:** n = 2

Despite of the progress in fields of chemical synthesis and engineered biosynthesis of antibiotics, the natural products have been the main sources of antimicrobial compounds.[39,40] The inhibitors of fatty acids synthase FabF and FabF/H, platensimycin (**16**) and platencin (**17**)**,** are two novel antibiotics that were reported recently from soil bacterial strains of *Streptomyces platensis*.[41] Platensimycin (**16**) had been isolated previously also in our group.[42]



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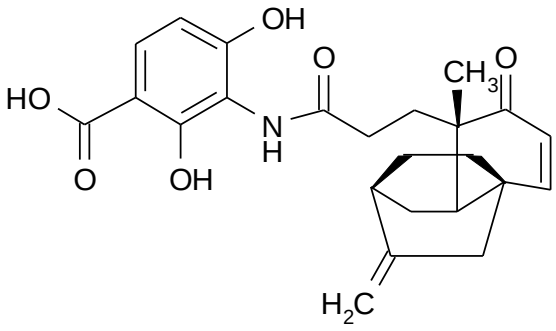


17

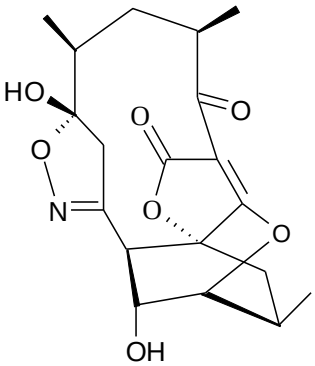
1.4 Marine Derived Anticancer Drugs

Oceans are covering more than 70% of the earth surface, but the marine environment as an important source for microbial products was nearly neglected in the past. After our group started respective investigations ca. 30 years ago as the first one in Europe, only recently, microbial metabolites isolated from different habitats of marine environment such as seawater, sediments, algae, sponges and different animals are gaining increasing importance.[43,44] Some chemical entities are playing a relevant role as autocrine cell regulators, regulators of the differentiation process.[45] An example is cancer, which is a major public health problem worldwide with millions of new cancer patients every year and many casualties resulting from this disease.[46] On the other side, actinomycetes supply by more than half of all discovered bioactive secondary metabolites. The secondary metabolites derived from marine actinomycetes are often having different types compared with those produced in terrestrial environments; they may posses different biological activities and have the potential to be developed as new leads for therapeutic agents.[47]

Compound	Source	Activity
Abyssomicins	*Verrucosispora* sp.	Antibacterial
Aureoverticillactam	*Streptomyces aureoverticillatus*	Anticancer
Bonactin	*Streptomyces* sp.	Antibacterial; antifungal
Caprolactones	*Streptomyces* sp.	Anticancer
Chandrananimycins	*Actinomadura* sp.	Antialagl; antibacterial; anticancer; antifungal
Chinikomycins	*Streptomyces* sp.	Anticancer
Chloro-dihydroquinones	*Novel actinomycete*	Antibacterial; anticancer
Diazepinomicin (ECO-4601)	*Micromonosproa* sp.	Antibacterial; anticancer; anti-inflammatory
3,6-disubstituted indoles	*Streptomyces* sp.	Anticancer
Frigocyclinone	*Streptomyces griseus*	Antibacterial
Glaciapyrroles	*Streptomyces* sp.	Antibacterial
Gutingimycin	*Streptomyces* sp.	Antibacterial
Helquinoline	*Janibacter limosus*	Antibacterial
Himalomycins	*Streptomyces* sp.	Antibacterial
IB-00208	*Actinomadura* sp.	Anticancer
Komodoquinone A	*Streptomyces* sp.	Neuritogenic activity
Lajollamycin	*Streptomyces nodosus*	Antibacterial
Marinomycins	'*Marinispora*'	Antibacterial; anticancer
Mechercharmycins	*Thermoactinomyces* sp.	Anticancer
MKN-349A	*Nocardiopsis* sp.	Unknown biological activity
Salinosporamide A (NPI-0052)	*Salinispora tropica*	Anticancer
Sporolides	*Salinispora tropica*	Unknown biological activity
Trioxacarcins	*Streptomyces* sp.	Antibacterial; anticancer; antimalarial

Table 2: Novel metabolites produced by marin actinomycetes during the period 2003-2005.[47]

The complex polyketides abyssomicins B (**18**), C (**19**) and D (**20**) were discovered in a rare actinomycete *Verrucosispora* strain by Süssmuth *et al*. The isolate from a sediment of the Japanese Sea exhibited good inhibitory activity against MRSA and VRSA (MIC 4 – 13 µg/ml), obviously by inhibition of the biosynthesis of *p*ABA.[48,49] Related secondary metabolites such as *ent*-homoabyssomicin A (**21**) and B (**22**) were isolated recently in our group from *Streptomyces* sp. Ank 212.[50]



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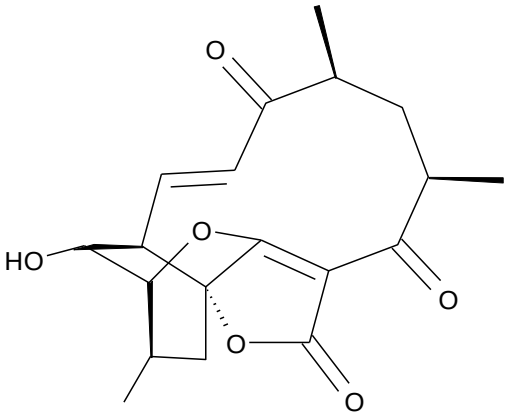

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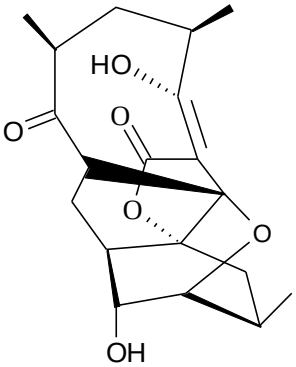

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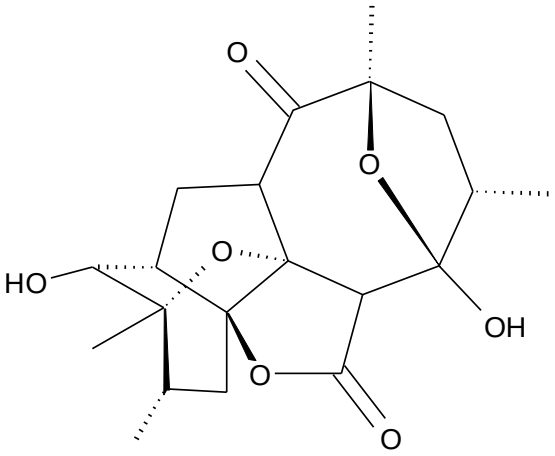

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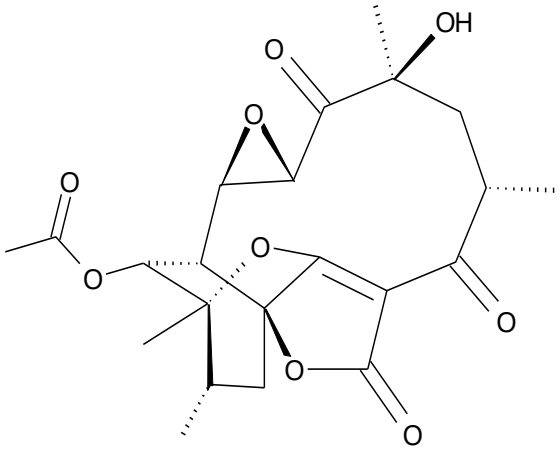
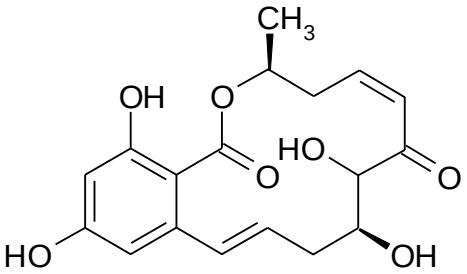


22

1.5 Fungi as a Source of Natural Products

Fungi are rich sources of structurally unique and biologically active secondary metabolites as well.[51] From a filamentous fungus MSX 63935 isolated from leaf litter collected in Nigeria, recently two new polyketides **23** and **24** were obtained. Compound **23** exhibited activity against three cancer cell lines MCF-7, H460, and SF268 and showed NF-κB inhibitory activity.[52]



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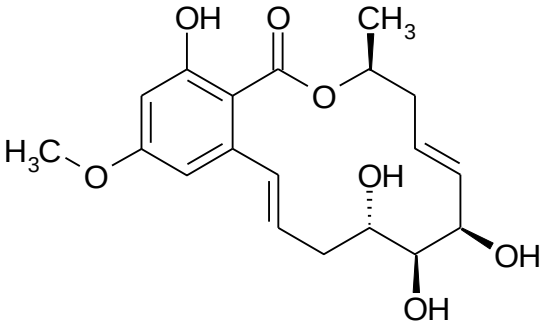

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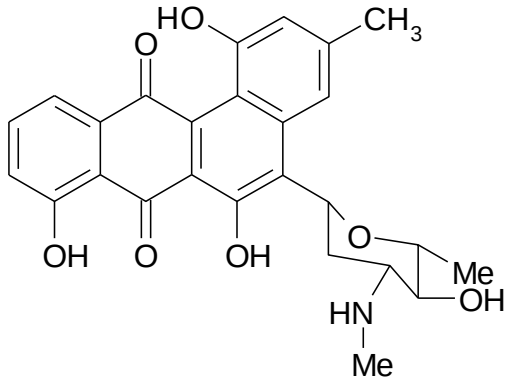
Aromatic polyketides are a further well-known group of antibiotics. Recent studies of *Streptomyces* sp. strain HB202, isolated from the marin sponge *Halichondria panicea*, afforded a benz[*a*]anthracene derivative named mayamycine (**25**)**,** which displayed potent cytotoxic activity against eight human cancer cell lines and exhibited activity against bacteria including antibiotic-resistant strains.[53] Fijiolide A (**26**) and B (**27**)**,** two chloroaromatic compounds related to Bergman cyclisation products

were isolated from a marine derived bacterium of the genus *Nocardiopsis*. Fijiolide A (**26**) inhibited TNF-R-induced NFκB activity by 70.3% in a dose-dependent manner with an IC_{50} value of 0.57 μM, while Fijiolide B (**27**) was less active and showed only 46.5% inhibition.[54]



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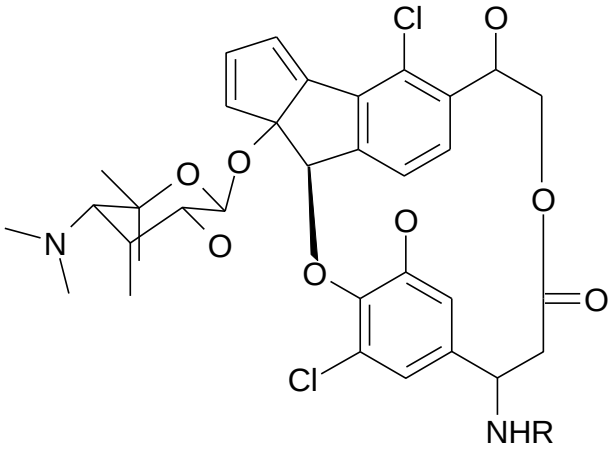
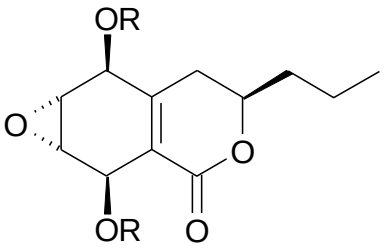


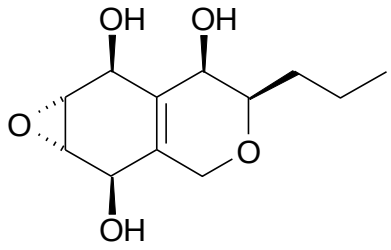
26: R = $COCH_3$
27: R = H

Endophytic fungi are a further rich source of bioactive compounds.[55,56] Endophytes are microorganisms, which live in the intercellular spaces of host plants without causing any visible signs of their presence.[57] Some of these internal colonizing microorganisms have an excellent potential to promote the plant growth [58], other endophytes are sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds.[59] The hosts of these microorganisms might be fungi[60], plants[61] and insects,[62] but also algae.[63] Endophytic fungi exhibited biological activity more often than those isolated from soil: 80% of the endophytic fungi from plants inhibited at least one of the test organisms, while only 64% of those from soils did so.[64]

Six new secondary metabolites were isolated from cultures of the endophytic fungus *Phomopsis* sp. isolated from the leaves of *Laurus azorica*, namely cycloepoxylactone (**28a, b**)**,** cycloepoxytriol A (**29**), cycloepoxytriol B (**30**) and phomolactones (**31a – c**)**.** Cycloepoxylactone (**28a**) has good antibacterial, antifungal, and algicidal activity against *Bacillus megaterium*, *Microbotryum violaceum*, and *Chlorella fusca*, respectively, whereas cycloepoxytriol B (**30**) had only good algicidal activity against *Chlorella fusca*.[65]



28a: R = H
28b: R = 4-Bromobenzoate



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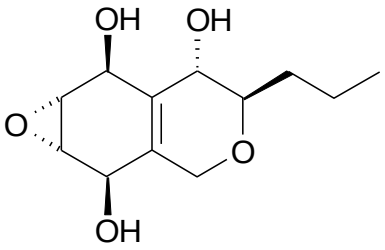

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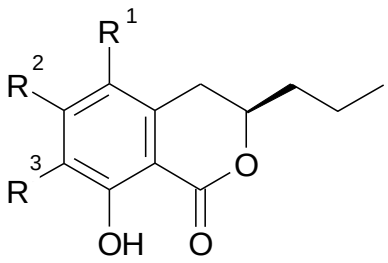
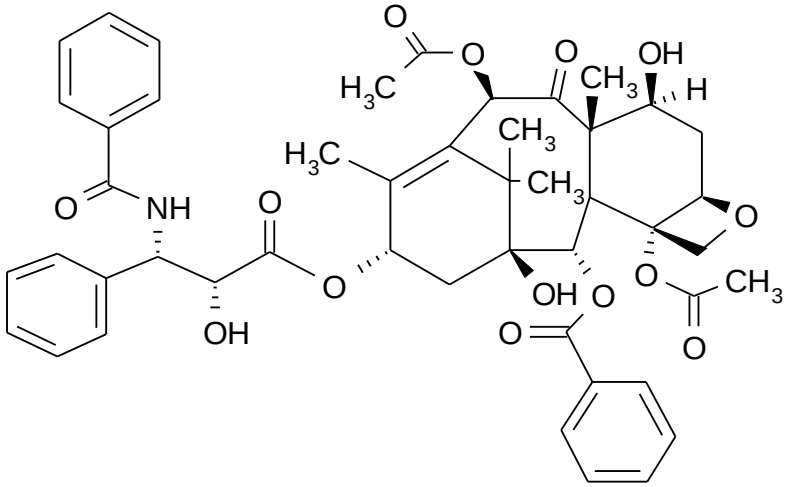


	R^1	R^2	R^3
31a	OH	Cl	H
31b	OH	H	H
31c	H	H	OH

The antiantileukemic and tumor inhibitory taxol (**32**) is an important example for products from endophytic microorganism. It was claimed to be formed by *Pestalotiopsis microspora*, an endophytic fungus isolated from *Taxus wallachiana*[66] and from the stem bark of the western yew tree *Taxus brevifolia*.[67] Taxol has numerous applications in medicine and is used against ovarian and breast cancer.[68]

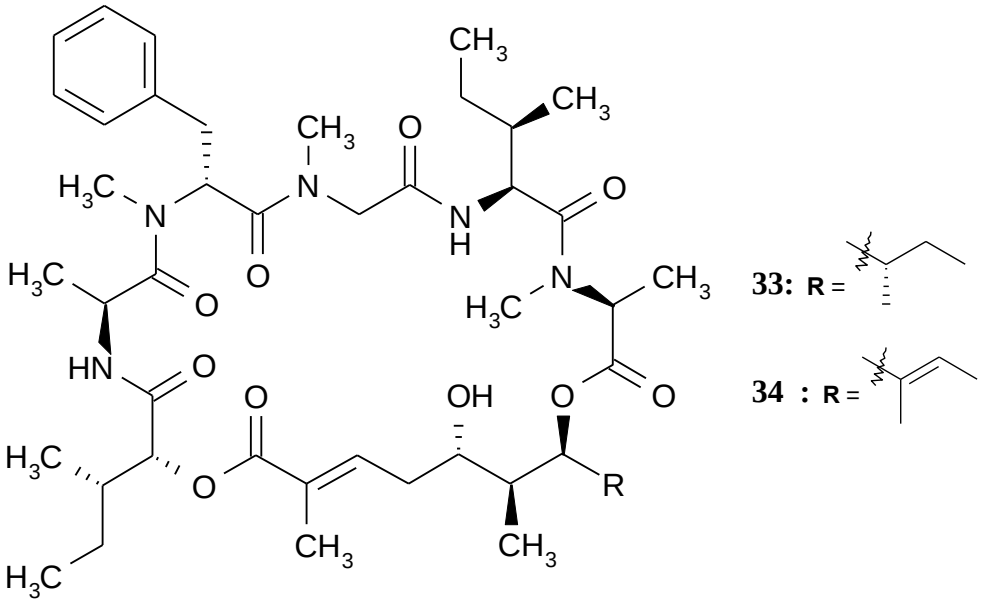


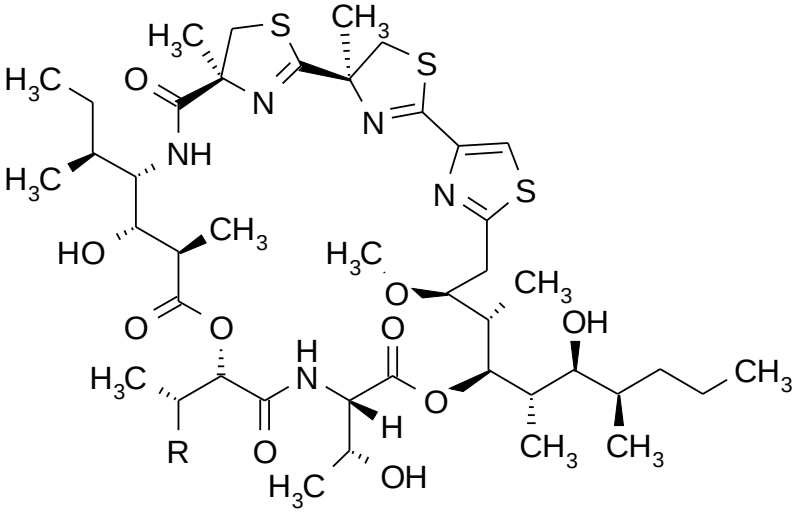
32

1.6 Cyanobacteria as a Source of Drugs

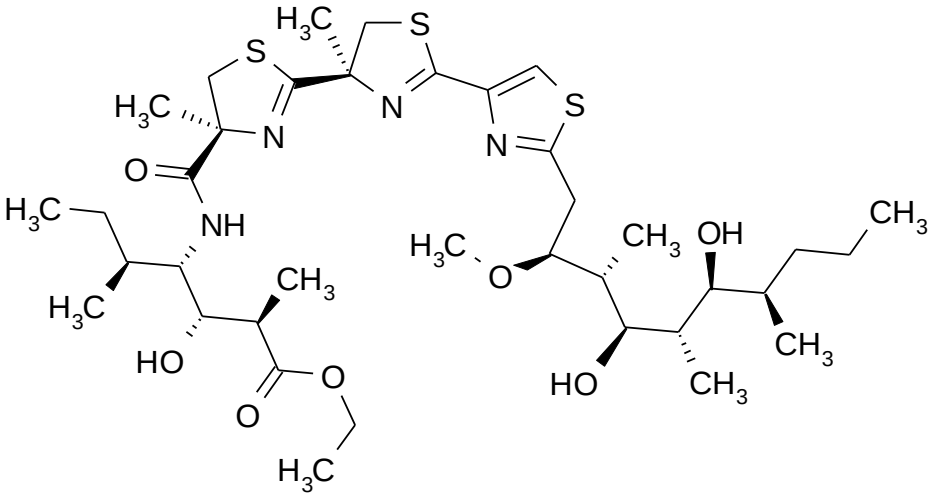
The discovery of majusculamides A and B by Moore in 1977[69] opened the door for biologists and chemist to focus on marine cyanobacteria, which were soon recognized to be prolific producers of structurally intriguing and biologically active secondary metabolites, many of which having toxic properties.[70] Secondary metabolites of marine cyanobacteria cover a variety of structure classes, including peptides, polyketides, terpenoids, and alkaloids. However, the most predominant structure classes are lipopeptides, which are formed by polyketide synthases (PKS) and nonribosomal peptide synthetases.[71-73]

Recently, the new cyclic depsipeptides lagunamide A (**33**) and B (**34**) were isolated from the marine cyanobacterium *Lyngbya majuscula*. Lagunamides A and B displayed significant antimalarial properties when tested against *Plasmodium falciparum*. They also possessed potent cytotoxic activity against P388 murine leukaemia cell lines; furthermore, these cyanobacterial compounds exhibited moderate antiswarming activities when tested against *Pseudomonas aeruginosa* PA01.[74] Two related peptide metabolites, the cyclic depsipeptide hoiamide B (**35**) and the linear lipopeptide hoiamide C (**37**) along with hoiamide A (**36**), were isolated from different collections of marine cyanobacteria obtained in Papua New Guinea. The depsipeptide **35** stimulated sodium influx and suppressed spontaneous Ca^{2+} oscillations, while the lipopeptide hoiamide C (**37**) had no significant effects in these assays.[75]





35: R = CH_3, **36**: R = H



37

2 Aim of Investigation

The main objective of the present investigation was concerned with the isolation and structure elucidation of new and preferably biologically active secondary metabolites from bacteria. This study was focussed mainly on the genus *Streptomyces* collected from terrestrial and marine sources.

To achieve this aim, chemical and biological screenings should be applied in such a way, that in a minimum of time a maximum of results is obtained. For this reason, a 'horizontal screening' should be applied: With a few biological tests using Gram-positive and Gram-negative bacteria, fungi and yeasts, microalgae and brine shrimps, the antibacterial, antifungal, phytotoxic and cytotoxic activities are covered, and results can be used as a lead for further detailed investigations. The established chemical screening should be applied additionally, extended by using an HPLC/MS screening.

The individual steps can be summarized as follows:

- *Microbiological part*: Collection of bacterial cultures from carefully selected habitats, preferably in cooperation with microbiologists. Optimisation of fermentation conditions and small-scale cultivations must be done before the upscaling to isolate the bioactive constituents.
- *Chemical part*: Crude extracts produced by fermentation must be separated using different chromatographic techniques (HPLC; Sephadex LH-20, silica gel, RP-18 column chromatography, etc). The obtained pure compound should be identified using spectroscopic method (NMR, MS, UV, IR) with the help of databases (AntiBase, Dictionary of Natural Products, Chemical Abstracts).
- Finally the pure compounds will be sent to bioassays (*i.e.* antimicrobial test or brine shrimp assay); also known metabolites might show new and interesting activities when tested against new targets.

3 General Techniques

3.1 Collection of Microbial Strains

Marine and terrestrial bacterial strains were obtained from different collaborations. The terrestrial *Streptomyces* spp. (code beginning with ANK) and AdM 9 were obtained from Prof. Dr. H. Anke, Institute for Biotechnology and Drug Research, Kaiserslautern, Germany. The terrestrial *Streptomyces* spp. (code beginning with WO) was obtained from Prof. Dr. Wolf. The terrestrial *Streptomyces* spp. (code beginning with GW) was obtained from the laboratory of Dr. Iris Grün-Wollny, Gießen. *Bacillus sp.* strain M 6 was obtained from Mr. Muaaz Al-Ajlani, Department of Microbiology and Molecular Genetics, Lahore, Pakistan. The marine derived *Streptomyces* spp. (code beginning with B) were obtained from Dr. E. Helmke, Alfred-Wegner-Institute for Polar and Marine Research, Bremerhaven, Germany. *Bacillus* spp. were isolated at the University of Braunschweig.

In a co-operation between our group in Göttingen and Dr. Mohamed Shaaban (National Research Centre), four Egyptian microbial strains were selected on the basis of their biological activity: The terrestrial *Streptomyces* sp. MH4 was isolated from Egyptian soil by Dr. Mohamed S. Abdelaziz, National Research Centre, Egypt. The fungi *Aspergillus oryzae (*isolated from rice halls) and a *Trichoderma* sp. were isolated by Dr. Mohammad Magdy El-Metwally, Soil and Water and Environment Research Institute, Egypt. The endophytic fungus *Aspergillus fumigatus* sp. isolate R7 was isolated from red leaves of Egyptian red sweat potatoes (*Ipomoea batatas*) by Dr. Mohsen S. Asker, National Research Centre, Egypt.

3.2 Work-up Procedure for Selected Microbial Strains

In the present study, the selection of promising bacterial strains producing biologically active and new secondary metabolites depends on two different types of assays: the biological and the chemical screening, which are combined in the so-called pre-screening.

3.3 Pre-screening

Initially, the strains were sub-cultured on agar plates for 3-7 days and microscopically examined for contaminations. Small pieces of the agar culture were then used

to inoculate 1 l Erlenmeyer flasks, each containing ~250 ml of medium, for a small-scale cultivation, followed by incubation on a rotary shaker at 28 °C. The culture broth was then lyophilised, and the dried residue was extracted with ethyl acetate. The obtained crude extract was used for the biological and chemical pre-screening.

3.4 Biological Screening

As there is still no general way to derive the biological activity of metabolites reliably solely from their structures, a multitude of pharmacological test systems has been developed. They can be subdivided into defined and complex systems: The defined systems are often using highly sophisticated receptor tests; they are often expensive, sometimes highly specific and therefore are giving low response rates, sometimes as low as 1: 20.000 and even 1: 80.000 (as in the case of platensimycin). Receptor tests are therefore less suitable for use in universities, where a quick, sensitive and affordable test is required. The selectivity should be low, so that broad ranges of activities can be covered; a response rate of 10-20 % is suitable. The resulting 'horizontal screening' can be realized best with microorganisms, i.e. by use of complex systems.

Based on agar diffusion method, the crude extracts are tested in our group against different microorganisms as shown in the next table. The tests with microorganisms are followed by assays for the brine shrimp toxicity, which is an indicator for potential anticancer activity.

Table 3: Some microbial species used for biological activity

Bacillus subtilis *Staphylococcus aureus* *Streptomyces viridochromogenes (Tü 57)*	Gram-positive
Mucor miehei *Candida albicans*	Fungi
Chlorella sorokiniana *Chlorella vulgaris* *Scenedesmus subspicatus*	Microalgae
Escherichia coli	Gram-negative

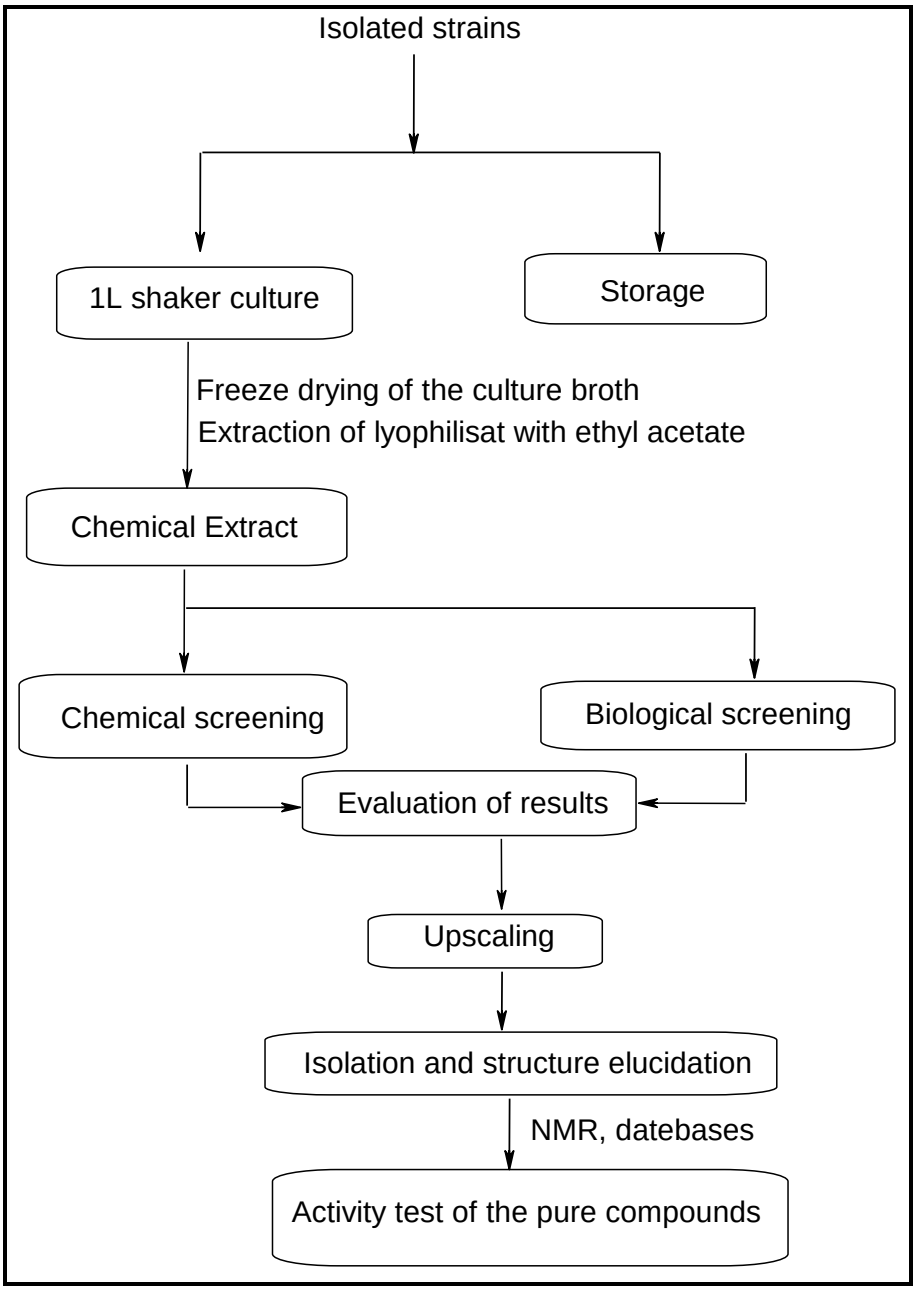


Figure 2: General screening of selected strains

3.5 Chemical Screening

The chemical screening is a method, which allows the detection of potentially interesting compounds at the earliest stages of separation. The TLC (thin layer chromatography) is a simple method used for the detection of bacterial constituents in the crude extracts. Compared with other methods like HPLC it is easy to perform, cheap and sufficiently reproducible. A spot of the crude extract is developed on a TLC plate with a CH_2Cl_2/CH_3OH solvent system. Then the developed TLC plate is visualized

under UV light and further localized by exposure to a suitable spray reagent. The following spray reagents are used in our group:

Anisaldehyde/sulphuric acid gives different colour reactions with many structural elements.

- Ehrlich's reagent is a specific reagent used to determine indoles and some other nitrogen containing compounds; indoles turn pink, blue or violet, pyrroles and furans become brown, anthranilic acid derivatives change to yellow.
- Concentrated sulphuric acid is especially used for polyenes. Short conjugated chains are showing a brown or black colour, carotenoids develop a blue or green colour.
- NaOH is used for the detection of *peri*-hydroxy-quinones, which turn red, blue or violet. The deep red prodigiosins are showing the colour of the yellow free base.
- The chlorine/*o,o'*-dianisidin reaction is used for the detection of peptides.

3.6 Large Scale Cultivation and Extraction

According to the pre-screening, the interesting strains were scaled up for further investigation. In some cases, in order to improve the microbial production of the interesting compounds, the optimisation of the culture conditions may be done. Well-grown agar sub-cultures were monitored and finally selected for performing the subsequent inoculation. A number of usually 100 Erlenmeyer flasks (1 l) each containing 250 ml culture medium (pH 7.8) were used for the inoculation and propagation of the bacteria on a linear shaker (28 °C). After four to five days, the culture broths were harvested, mixed with Celite (diatomaceous earth as filter aid) and filtered under pressure (filter press). The latter step is necessary to separate the water phase, which was successively adsorbed on Amberlite adsorption resin XAD-16 and the latter finally extracted with methanol. The biomass remaining after filtration was exhaustively extracted with ethyl acetate and acetone. Finally, the crude extracts were evaporated under vacuum and tightly kept for subsequent chromatographic work.

According to the amount of the obtained crude extracts, a suitable isolation method will be determined, with respect to the polarity of the compounds of interest. Extracts from water phase and cell mass are combined, if the composition is similar; otherwise they are separated independently. For separation of accompanying fats, the crude extract is firstly defatted by cyclohexane, and then the crude extracts are subjected to a silica gel column, which is normally eluted with a stepwise gradient of dichloromethane/methanol. Since silica gel is acidic, some compounds may be rearranged, oxidised, cleaved, or destroyed. It is better therefore to use the size exclusion chromatography on Sephadex LH-20 for further separations. The latter has the advantage of a high recovery rate as well as to minimize the destruction of labile compounds. Further purification of the fractions can be done with the aid of HPLC on RP-18 columns.

3.7 Dereplication

The development of screening steps for the discovery of biologically active compounds has resulted in a need to prioritise those samples, which are deemed 'active'. When these samples are derived from different natural product sources, it is estimated that it takes $50.000 and 3 months of work to isolate, identify and elucidate such an active compound and there will be a distinct sense of 'wasted' effort if the isolate is a well-known compound [76]. So the term *dereplication* is commonly used in the natural products community to define the complementary processes of rapid identification of natural compounds. For this reason, a number of techniques have been adopted.

The comparison of UV as well as mass data in combination with HPLC retention times of compounds from our own HPLC/MS database is an efficient method. The advantage of this method is that a low amount of the sample or crude extract is sufficient.

The identification of new compounds can be managed by determination of the molecular weight and the fragmentation pattern by MS as well as the chromophore of the respective compound.

An HPLC-UV-ESI-MS/MS database with over 600 compounds has been established in our group for dereplication on the basis of crude extracts. The identification

of a given component could be accomplished by fitting of the obtained data with reference spectra and also by comparison with related compounds, which have the same chromophore or aglycone.

In our group we have access to the important leading databases The Dictionary of Natural Products (Chapman & Hall), AntiBase (Chemical Concepts) and the Chemical Abstracts. The Dictionary of Natural Products (DNP) collects nearly all-natural products, especially plant metabolites. The disadvantage of DNP is the lower search capability as well as limited spectroscopic information.

On the other hand, AntiBase is more practical, efficient and advanced for the dereplication of microbial metabolites. More than 38.000 compounds from microbial sources are included, which can be searched by substructures and a wide range of further search capabilities.

The identification of known compounds can easily be done step by step by comparison of ^{1}H NMR data and molecular weights according to high resolved masses. The most important advantage of AntiBase is that it offers an access to the ^{1}H NMR data of many and the ^{13}C NMR data for nearly all compounds with known structure. The most comprehensive worldwide database is the Chemical Abstracts; normally a search in CA is the final step to confirm the novelty of a compound.

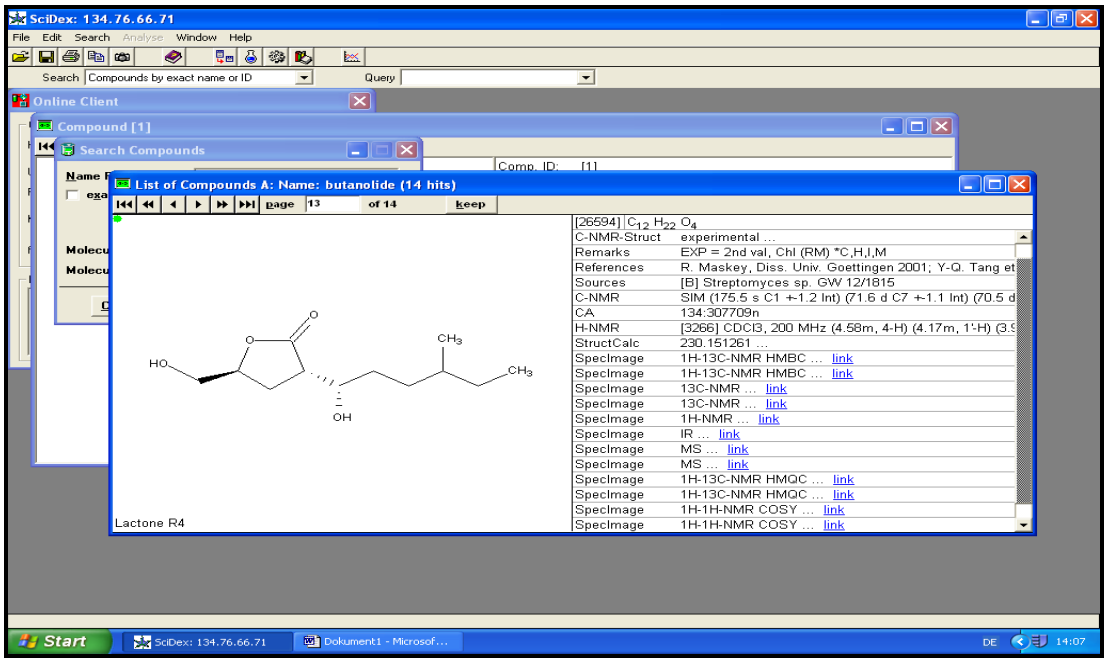


Figure 3: AntiBase, a Data Base for Rapid Dereliction and Structure Determination of Microbial Natural Products[77]

4 Investigation of Selected Microbial Strains

4.1 Terrestrial *Streptomyces* sp. ANK 264

The terrestrial *Streptomyces* sp. ANK 264 was selected according to the chemical and biological screening. The TLC analysis exhibited blue UV florescent zones, which showed different colour reactions with anisaldehyde/sulphuric acid and Ehrlich's reagent. The crude extract showed good biological activities in the agar diffusion test against different microorganisms as mentioned in Figure 248.

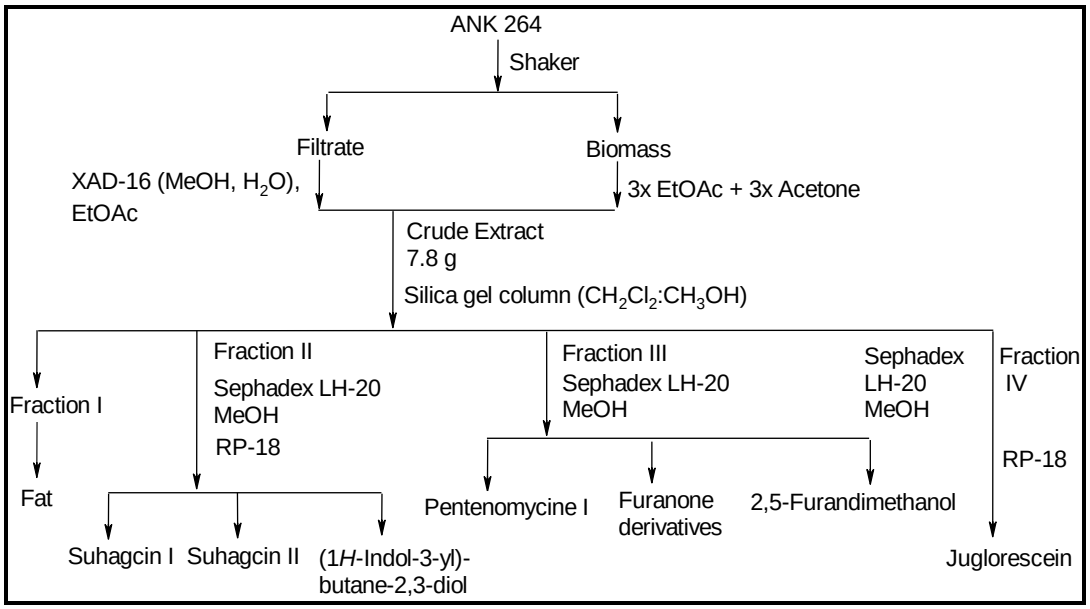


Figure 4: Work-up scheme of terrestrial *Streptomyces* sp. ANK 264

4.1.1 Juglorescein

Fraction IV exhibited compound **38** in the polar region as UV absorbing band, which turned to green by spraying with anisaldehyde/sulphuric acid and heating. P*seudo*molecular ions were found at 607 $[M+Na]^+$, 1192 $[2M+Na]^+$ Dalton by ES-IMS. HRESIMS gave the molecular formula as $C_{28}H_{24}O_{14}$. The ^1H NMR spectrum displayed a broad 1H downfield singlet of a chelated hydroxyl group at δ 12.33. It showed in the aromatic region four doublet of doublet signals at δ 7.45, 7.34, 7.27 and 6.88, two triplets were visible at δ 7.69 and 7.54; this pattern in the aromatic region suggested two 1,2,3-trisubstituted benzene ring. In addition two broad signals for H/D exchangeable protons appeared at δ 6.78 and 6.61. In the aliphatic region two oxymethines were visible at δ 4.68 and 4.42. In addition to two methine groups

at δ 3.37 (t), 3.23 (d), one further methylene group was observed as ABX systems at δ 3.07 and 2.17. The downfield shift indicated a connection with sp^2 carbons or heteroatoms. Finally two methylene groups in a ring were observed at δ 2.71-2.59.

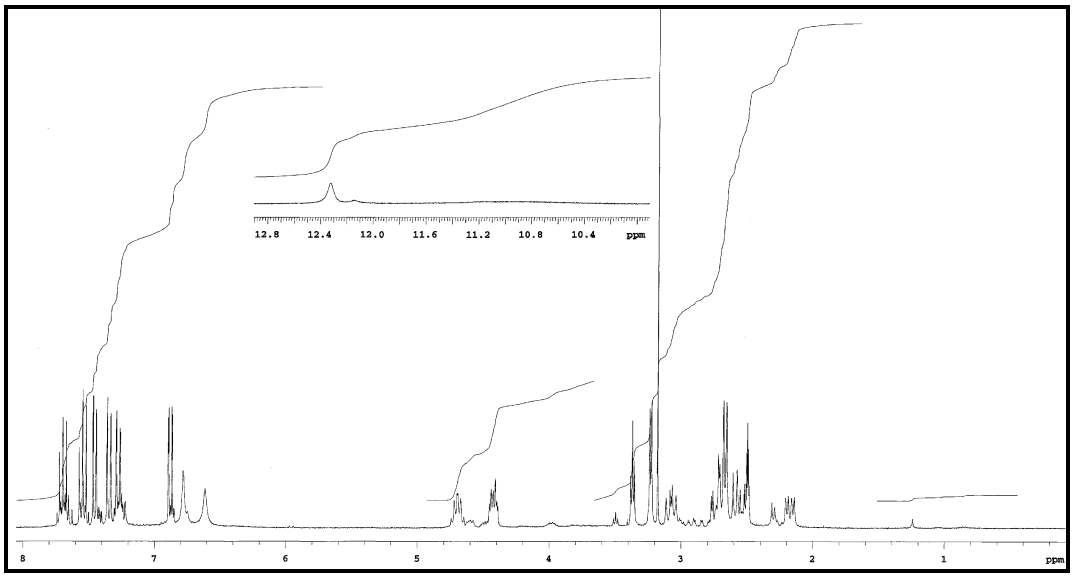


Figure 5: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of juglorescein (**38**)

The HSQC and ^{13}C NMR spectra displayed 28 carbon signals confirmed by HRESIMS. Among them three ketone carbons were observed at δ 206.7, 204.0, 194.0, in addition to 12 aromatic quaternary or methine carbons. The other signals were in the upfield region.

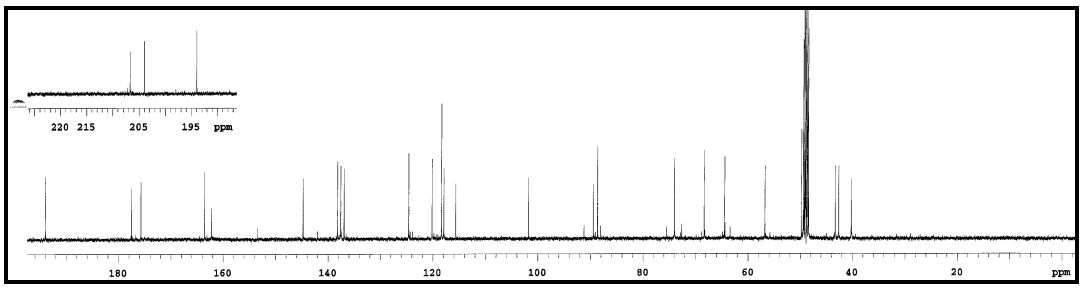


Figure 6: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of juglorescein (**38**)

The ^{1}H,^{1}H COSY spectrum showed two 1,2,3-trisubstituted benzene rings **A**, **B**. In addition, two substituted alkyl chains **C**, **D** were visible.

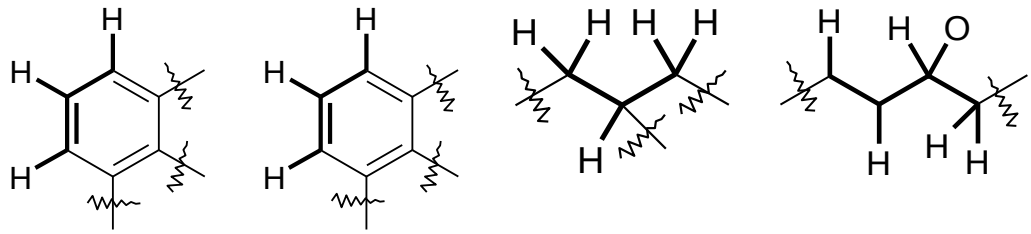


Figure 7: selected correlations ^{1}H,^{1}H COSY (**—**) of juglorescein (**38**)

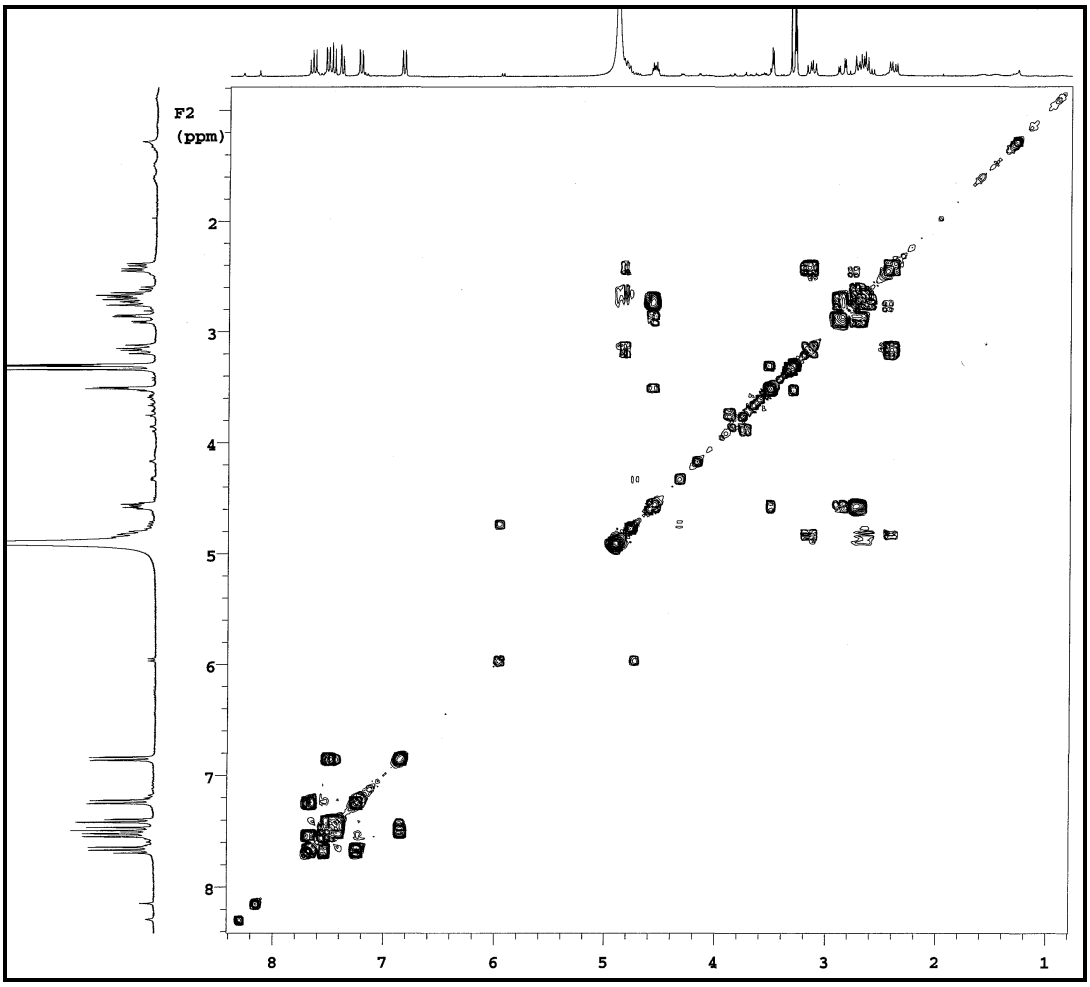


Figure 8: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of juglorescein (**38**)

HMBC date were used to connect these fragments and to get the final structure: The proton at δ 7.45 (δ_c 120.2) showed a ^{3}J correlation with the carbonyl at δ 194.0. The latter showed a strong coupling with the proton at δ 3.37 (δ_c 49.9), the proton at δ 2.71 (Ha-11) showed a ^{3}J correlation with the carbonyl at δ 177.6. On the other hand, the proton at δ 3.23 (H-3') exhibited ^{3}J coupling with an oxymethine carbon at δ 68.3 (C-10) and a ketone carbonyl at δ 206.7 (C-4'), which was confirmed by a ^{3}J correlation with the methine proton at δ 3.37 (δ_c 49.9). In addition, the proton at δ

2.17 (Hb-9') showed a ^{3}J coupling with the quaternary carbons at δ 89.5 (C-3), 64.5 (C-2'), 56.8 (C-3') and a hemiketal carbon at 101.8 (C-1'), which finally showed a strong cross signal with the proton at δ 7.34.

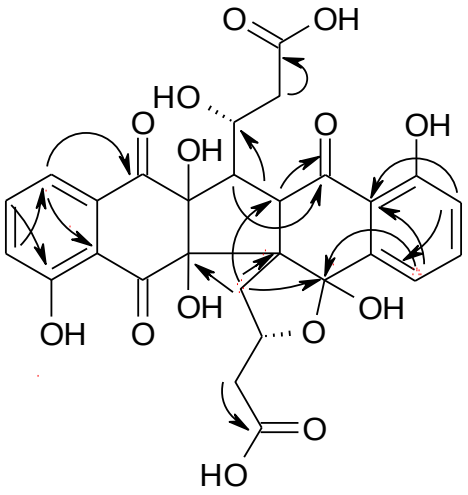
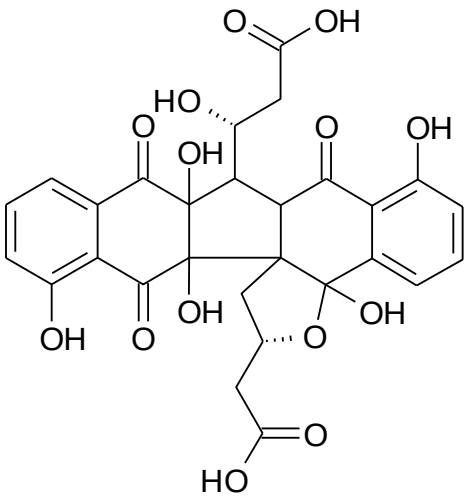
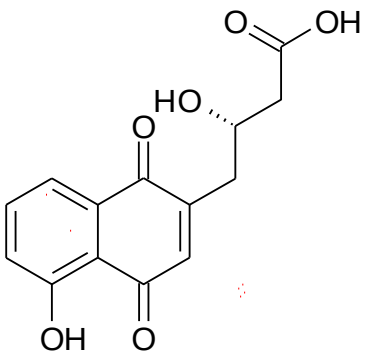


Figure 9: HMBC (→) couplings of juglorescein (**38**)

A search in AntiBase[77] supported by ^{1}H, ^{13}C NMR, 2D and MS spectroscopic data led to juglorescein, which was further confirmed by the literature [78] and by comparison with authentic spectra [79]. Juglorescein (**38**) is a dimer of juglomycin C (**39**) with a five-membered ring between the two monomeric moieties. In juglorescein (**38**), two juglomycin C (**39**) units are connected together by C,C and C,O bonds, forming a central isochroman or a chroman system. Juglorescein (**38**) showed no activity in the antimicrobial tests.[78]



38



39

Table 4: ^{1}H and ^{13}C NMR shifts of juglorescein (**38**)

Position	δ_C [a, b]	mult.	δ_H [a, c] (mult., *J* in [Hz])
1	194.0	C_q	-
2	88.7	C_q	-
3	89.5	C_q	-
4	204.0	C_q	-
4a	118.4	C_q	-
5	163.6	C_q	-
6	124.6	CH	7.27 (dd, 8.4, 1.1)
7	138.2	CH	7.69 (t, 7.5)
8	120.2	CH	7.45 (dd, 7.5, 1.1)
8a	137.0	C_q	-
9	49.9	CH	3.37 (t, 4.3)
10	68.3	CH	4.42 (m)
11	43.3	CH_2	2.71 (d, 3.39) overlapped in 2.67- 2.59 (m)
12	177.6	C_q	-
1'	101.8	C_q	-
2'	64.5	C_q	-
3'	56.8	CH	3.23 (d, 4.4)
4'	206.7	C_q	-
4'a	115.7	C_q	-
5'	162.2	C_q	-
6'	117.9	CH	6.88 (dd, 8.3, 1.1)
7'	137.6	CH	7.54 (t, 7.8)
8'	118.4	CH	7.34 (dd, 7.8, 1.1)
8'a	144.8	C_q	-
9'	40.2	CH_2	3.07 (m)
			2.17 (dd, 13.8, 6.1)
10'	74.0	CH	4.68 (m)
11'	42.6	CH_2	2.67- 2.59 (m)
12'	175.8	C_q	-
OH	-	-	12.33 (s br)
OH	-	-	6.61 (s br)
OH	-	-	6.78 (s br)

[a] DMSO-d_6; [b] 125 MHz; [c] 300 MHz

4.1.2 (1*H*-Indol-3-yl)-butane-2,3-diol

(1*H*-Indol-3-yl)-butane-2,3-diol (**40**) isolated from middle polar fraction as colourless oily substance turned to red with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **40** exhibited five protons in the aromatic region, which were indicative for a 3-substituted indole moiety. The spectrum displayed two doublets of doublets at δ 7.55 and 7.30 and two triplets of doublets at δ 7.06 and 6.97, suggesting a 1,2-disubstituted benzene ring. In addition there was a singlet at δ 7.09. In the aliphatic region, two methine protons attached to a heteroatom overlapped at δ 3.68. In addition two diastereotopic methylene protons at δ 3.03 and 2.80 were visible, which indicated the neighbourhood of a stereogenic centre and possibly an sp^2 carbon or a heteroatom. Finally, a methyl group gave a doublet at δ 1.20.

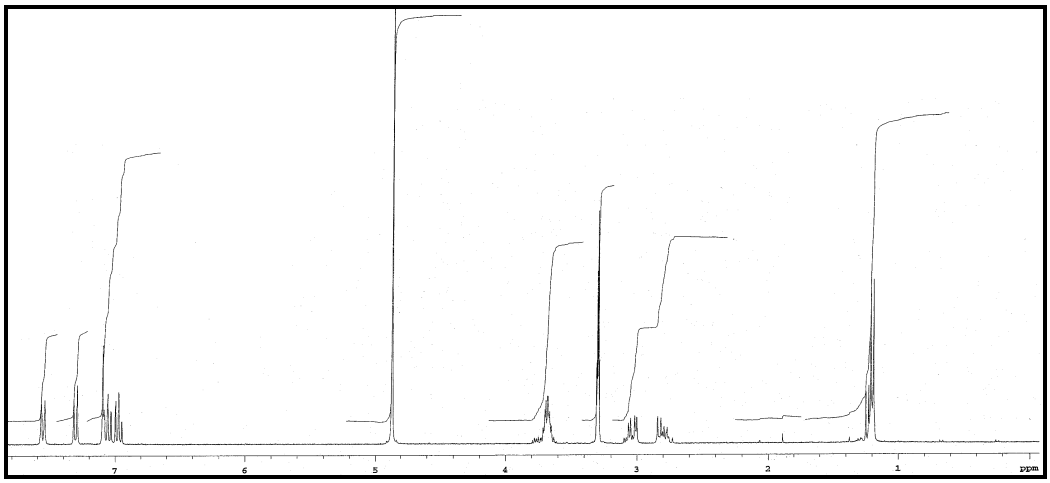
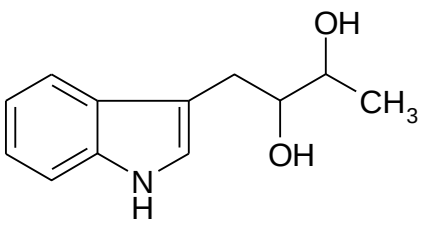


Figure 10: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of (1*H*-indol-3-yl)-butane-2,3-diol (**40**)

The molecular weight of compound **40** determined as 205 from the *pseudo*molecular ions at 228 $[M+Na]^+$, 433 $[2M+Na]^+$, 204 $[M-H]^-$ and 409 $[2M-H]^-$ Dalton, determined by ESIMS. A search in AntiBase[77] with these data led to (1*H*-indol-3-yl)-butane-2,3-diol (**40**). It was further confirmed by the literature [80]



40

4.1.3 Deferrioxamine E

Deferrioxamine E (**41**) was isolated as a white solid which gave a pink colour on spraying with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of compound **41** displayed two H/D exchangeable protons at δ 9.26 and 7.38. In the aliphatic region four methylene groups were observed, two attached to heteroatoms at δ 3.50 (t) and 3.05 (q), the others appeared at δ 2.61 (t) 2.33 (t); finally three methylene groups at δ 1.55 (m) 1.42 (m), 1.26 (m) were visible.

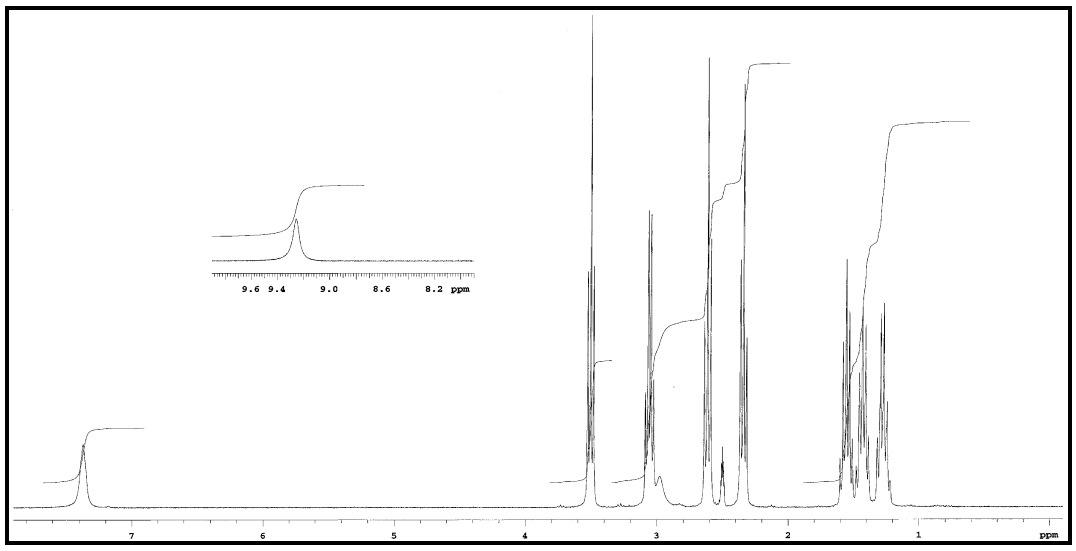


Figure 11: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of deferrioxamine E (**41**)

The HSQC and ^{13}C NMR spectra exhibited 8 signals, among them two carbonyl signals of acid, ester or amide groups overlapped at δ 171.1. Further seven methylene groups were visible.

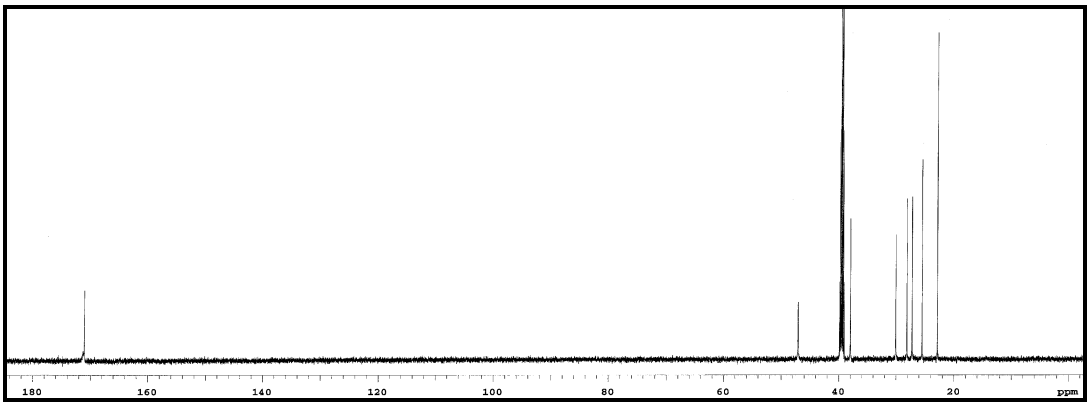


Figure 12: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of deferrioxamine E (**41**)

In the $^{1}H,^{1}H$ COSY spectrum of compound **41**, the methylene group at δ_H 3.50 correlated with a second methylene at δ_H 1.55, which for his part showed a strong correlation with the methylene signal at δ_H 1.26. The later gave a cross signal with a methylene group at δ_H 1.42. This one showed finally a ^{3}J correlation with the methylene at δ_H 3.05 (fragment A). The methylene group at δ_H (2.61) showed a ^{3}J correlation with the methylene at δ_H 2.33 (fragment B)

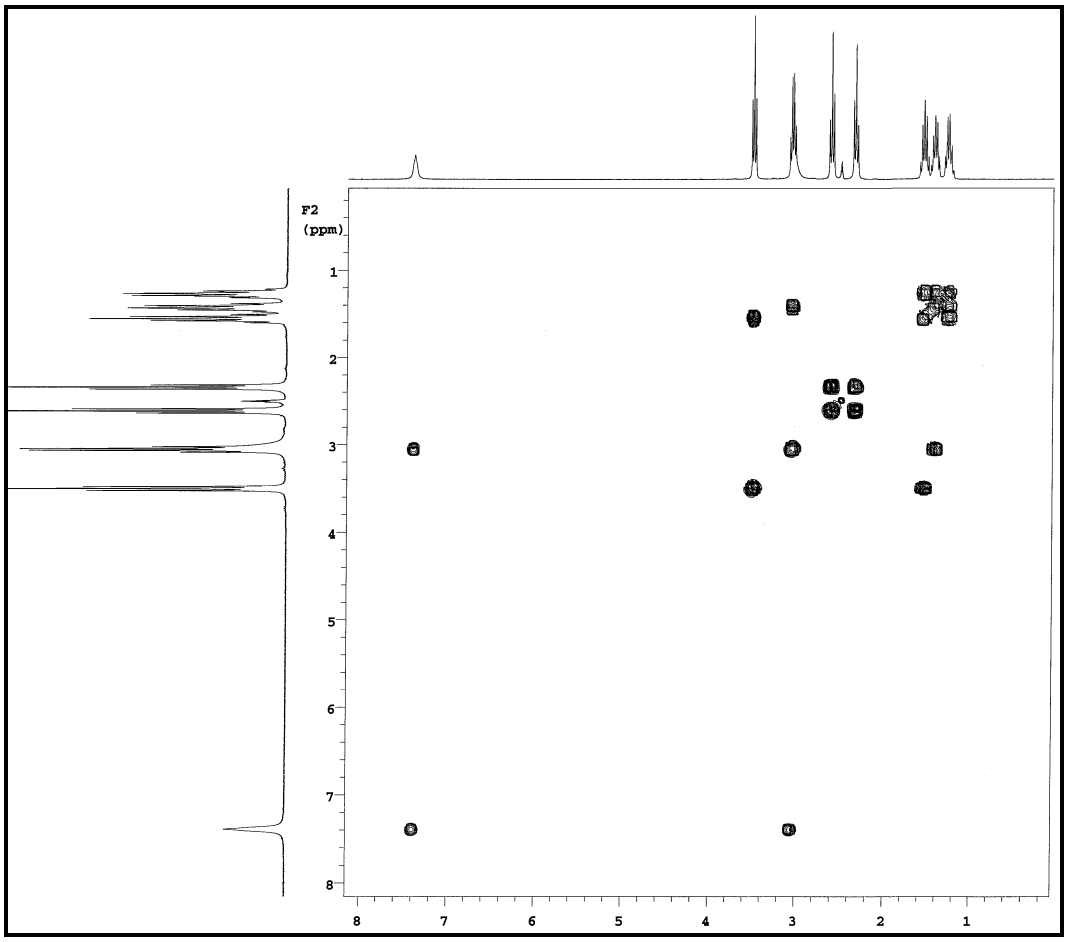
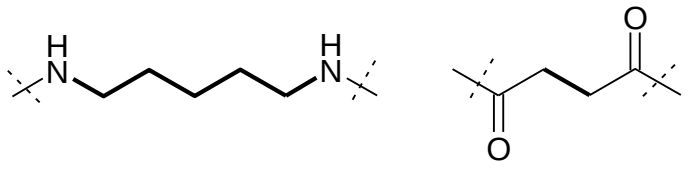


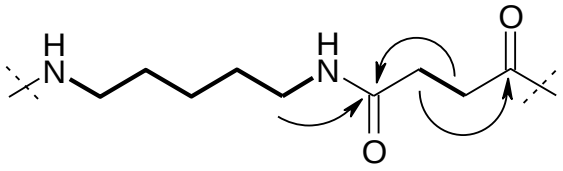
Figure 13: $^{1}H,^{1}H$ COSY spectrum (DMSO-d_6, 500 MHz) of deferrioxamine E (**41**)



A **B**

Figure 14: correlations $^{1}H,^{1}H$ COSY (▬) of substructures of deferrioxamine E (**41**)

By means of the HMBC spectrum these two fragments were connected via an amide bond to give the fragment **C.**



C

Figure 15: Selected HMBC (→) and $^{1}H,^{1}H$ COSY (▬) correlations of deferrioxamine E (**41**)

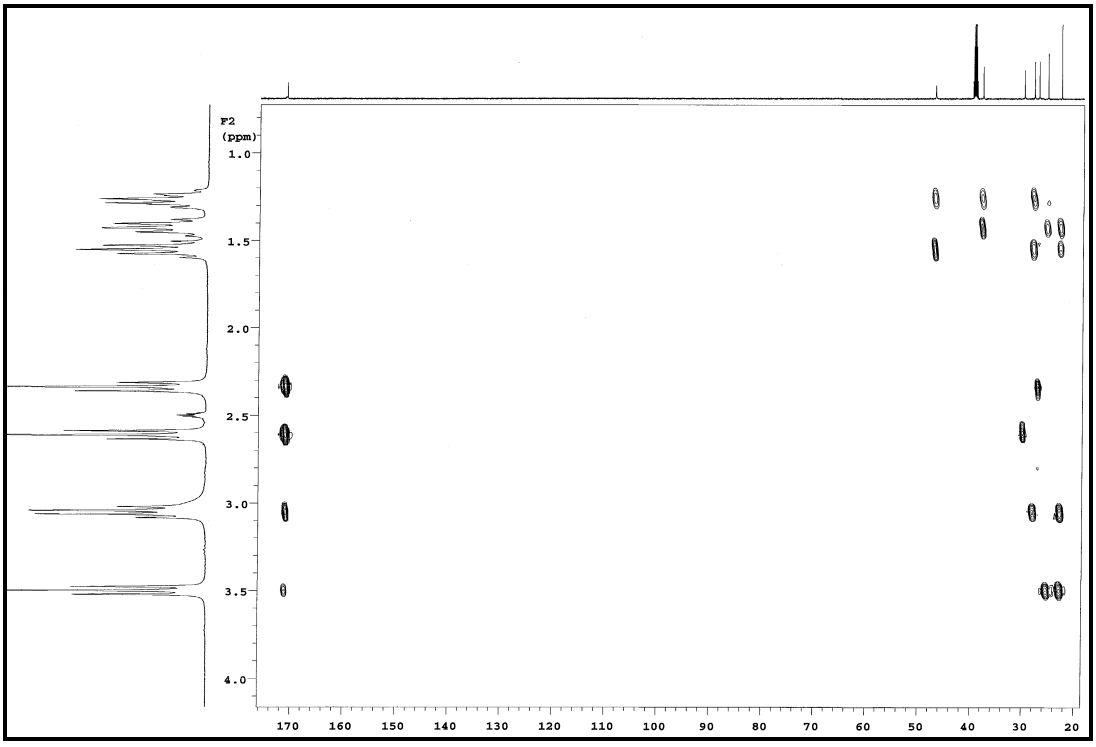
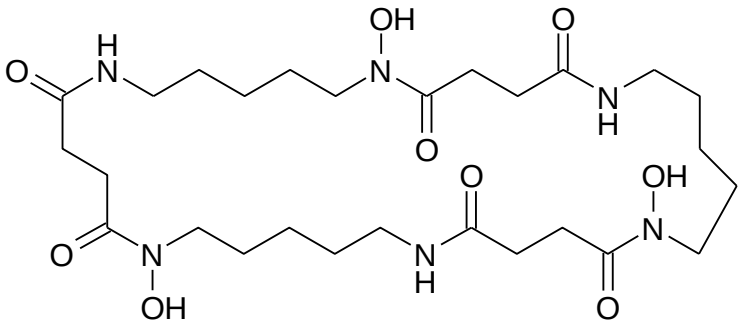


Figure 16: HMBC spectrum (DMSO-d_6, 500 MHz) of deferrioxamine E (**41**)

Low resolution ESI MS revealed a *pseudo*molecular ion peak at *m/z* 601$[M+H]^+$, which fixed the mass as 600 Dalton. A search in AntiBase with substructure C and the mass led to deferrioxamine E (**41**). It was further confirmed by the literature values[81] and comparing with authentic spectra.



41

Deferrioxamine E (**41**) and the smaller ring of bisucaberin are giving positive reactions (violet colour) with peptide reagent (chlorine/*o*-dianisidin reaction) due to the presence of the hydroxamate moiety [CO-N(OH)] in the structure;[81] it also chelates ferric ions (Fe^{3+}) very strongly. The iron chelators deoxynocardamine and deferrioxamine E (**41**) were previously described from the culture broth of an actinomycete of the genus *Streptomyces* isolated from an unidentified marine sponge.[82]

4.1.4 Pentenomycin I

Pentenomycin I (**42**) was isolated as colourless solid from fraction III as slightly UV active compound. The ^{1}H NMR spectrum displayed two 1H doublets at δ 7.64 and 6.25, which is indicative for an α,β-unsaturated carbonyl compound. An oxymethine group at δ 4.75 and diastereotopic methylene protons at δ 3.68 and 3.55 were visible, which indicated the neighbourhood of a stereogenic centre and possibly an sp^2 carbon or heteroatom.

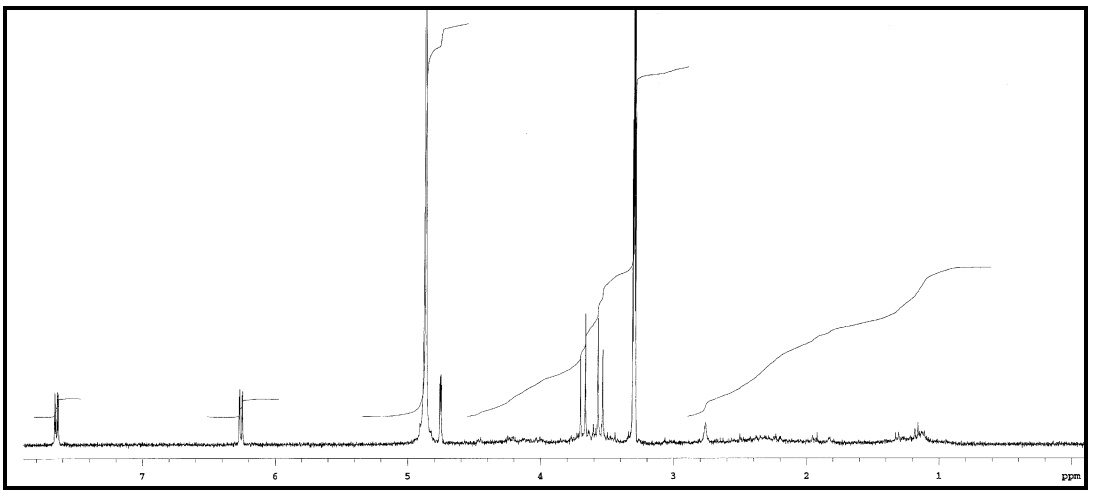


Figure 17: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of pentenomycin I (**42**)

The ^{13}C NMR spectrum of **42** exhibited 6 carbon signals, among them a ketocarbonyl at δ 208.7. In the sp^2 region, α,β-unsaturated carbons at δ 164.2 and δ 134.3 conjugated with the carbonyl were observed. Moreover, a quaternary carbon at δ 76.3 and another aliphatic methine at δ 73.2 were observed. Finally, a methylene group at δ 64.4 connected with a heteroatom was visible.

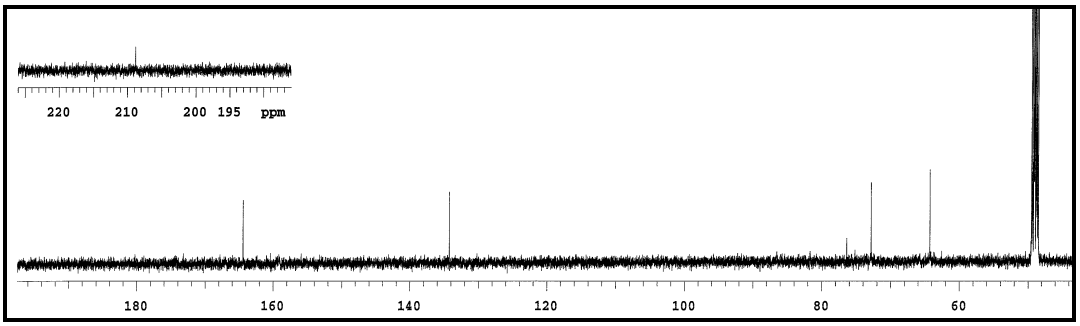
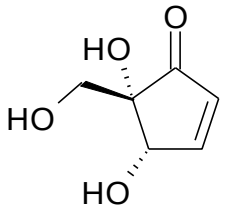


Figure 18: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of pentenomycin I (**42**)

A search in AntiBase supported by ^{1}H and ^{13}C NMR spectroscopic data led to pentenomycin I (**42**). It was further confirmed by the literature.[83] Pentenomycin I was moderately active against gram-positive and gram-negative bacteria.[84]



42

4.1.5 5-Hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2-(5*H*)furanone

Furanone **43** was isolated from fraction III as colourless oily substance; it gave with anisaldehyde reagent a blue colour. The ^{1}H NMR spectrum displayed in the olefinic region a 1H singlet at δ 5.87 of an anomeric proton. In addition, in the aliphatic region, a doublet of an oxymethine was observed at δ 4.15. Moreover, a methyl singlet connected to an sp^2 carbon appeared at δ 2.08. The latter methyl signal overlapped with a methine group at δ 2.03. In addition, two methyl doublets of an isopropyl unit were observed at δ 1.00 and 0.84.

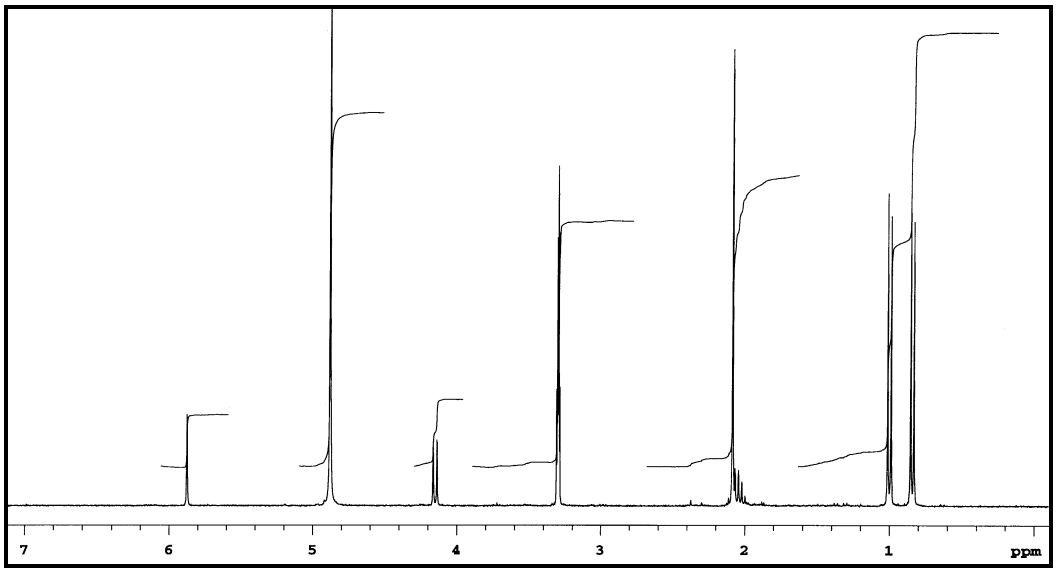


Figure 19: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 5-hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2(5*H*)furanone (**43**)

In ^{13}C NMR spectrum exhibited 9 carbon signals, among them a quaternary carbon at δ 173.1 for the carbonyl of an acid, ester or amid. Two quaternary carbons in the sp^2 region at δ 160.5 and 131.4, and an anomeric carbon at δ 100.7 were visible. Moreover, an oxymethine carbon was observed at δ 72.6. In addition, a methyl carbon at δ 12.0 and an isopropyl unit were observed at 34.2, 19.3, 18.9.

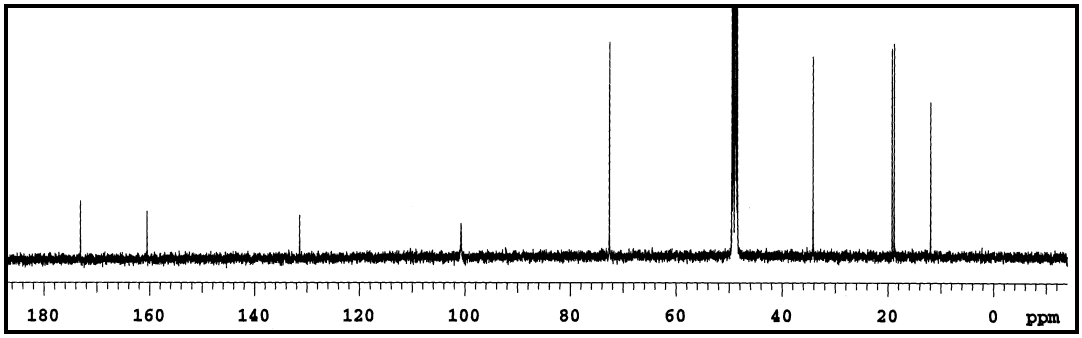
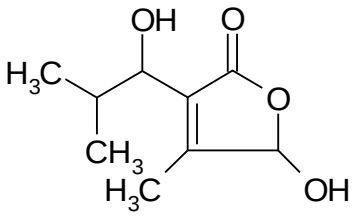


Figure 20: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 5-hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2-(5*H*)furanone (**43**)

A search in AntiBase with these ^{1}H and ^{13}C NMR data led to 5-hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2-(5H)furanone (**43**). The structure was further confirmed by comparison with the literature.[86]



43

The lactone **43** showed antibiotic activity against *Pseudomonas aeruginosa* with weak inhibition of the chitinase from *Serratia marcescens*.[85] Also, this lactone was synthesized by Grossmann *et al*.[86]

4.1.6 Suhagcine I

Suhagcine I (**44**) was isolated as colourless solid, which showed UV absorbance band at 254 nm on TLC plate and was visualized by a blue colour by spraying with anisaldehyde/sulphuric acid and heating. The molecular formula of suhagcine I (**44**) was established as $C_{14}H_{22}O_5$ by HRESIMS. The ^{1}H NMR spectrum of suhagcine I (**44**) showed three olefinic proton signals at δ 6.81 (t, H-7), 5.69 (m, H-11) and 5.50 (m, H-10), two overlapped oxymethine signals at δ 4.18 (m, H-3, 9), one methine signal at δ 3.43 (m, H-4) and two methylene signals at δ 2.48 (m, H-8) and 2.33 (m, H-2). In addition, one methyl singlet and two methyl doublets at δ 1.76 (H-14), 1.69 (H-12) and 1.11 (H-13) were observed.

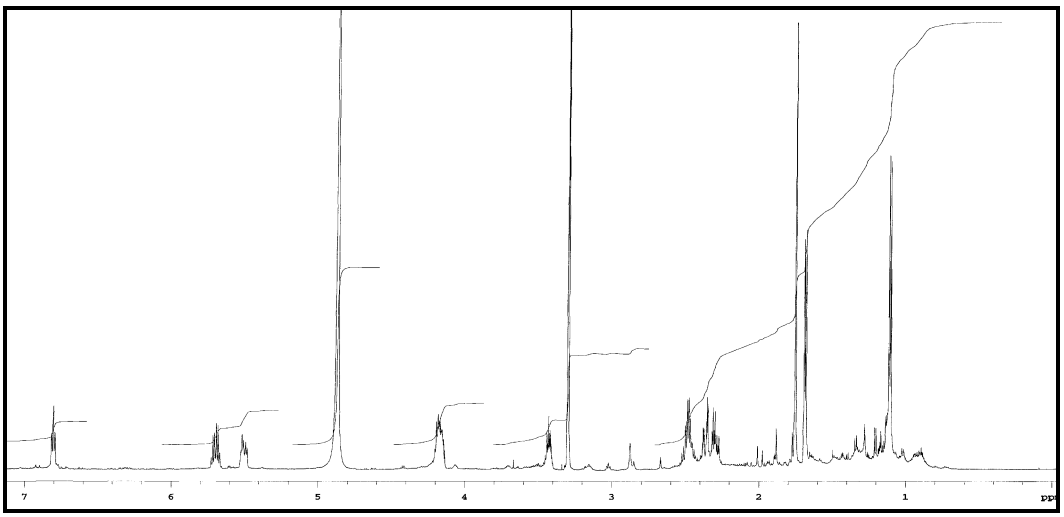


Figure 21: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of suhagcine I (**44**)

The ^{13}C NMR spectrum of suhagcine I (**44**) exhibited 14 carbon signals. Among them, a ketone carbonyl at δ 206.5, the carbonyl of an acid, ester or amide at δ 175.2,

four sp^2 carbon signals (three methine and one quaternary), two oxymethine signals at δ 72.5 and 71.1, one methine signal at δ 45.7, two methylene at δ 41.1 and 38.2, three methyl signals at δ 17.9, 14.1 and 11.9 were determined according to the HSQC spectrum.

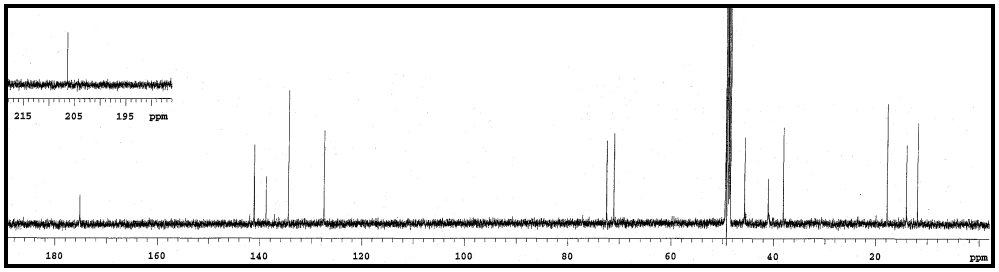
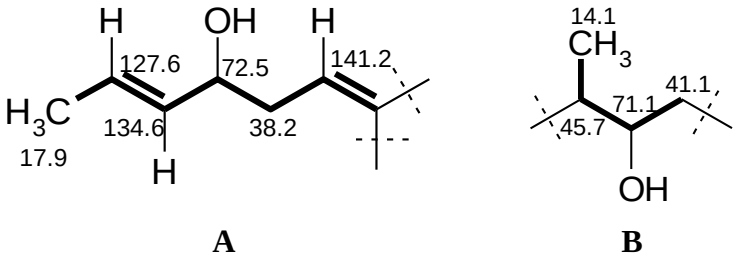


Figure 22: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of suhagcine I (**44**)

Most of the connectivities in suhagcine I (**44**) were established by COSY correlations. The olefinic proton at δ 5.50 (134.6) correlated to another olefinic proton at δ 5.69 (127.6) and an oxymethine proton at δ 4.18 (72.5). The latter correlated again with the methylene protons at δ 2.48 (38.2), which in turn again correlated with the olefinic proton at δ 6.81 (141.2). The methyl doublet at δ 1.69 (17.9) showed correlation with an olefinic proton at 127.6. According to the COSY data, the substructure **A** was drawn. In addition, one oxymethine proton at δ 4.18 (71.1) showed correlations to the methine proton at δ 3.43 (45.7) and methylene protons at δ 2.33 (41.1). The methyl doublet at δ 1.11 (14.1) showed correlation to the methine proton at δ 3.43 (45.7). From these data, the second substructure **B** could be constructed.



A **B**

Figure 23: selected $^1H,^1H$ COSY (▬) correlations of substructure **A**, **B** in suhagcine I (**44**)

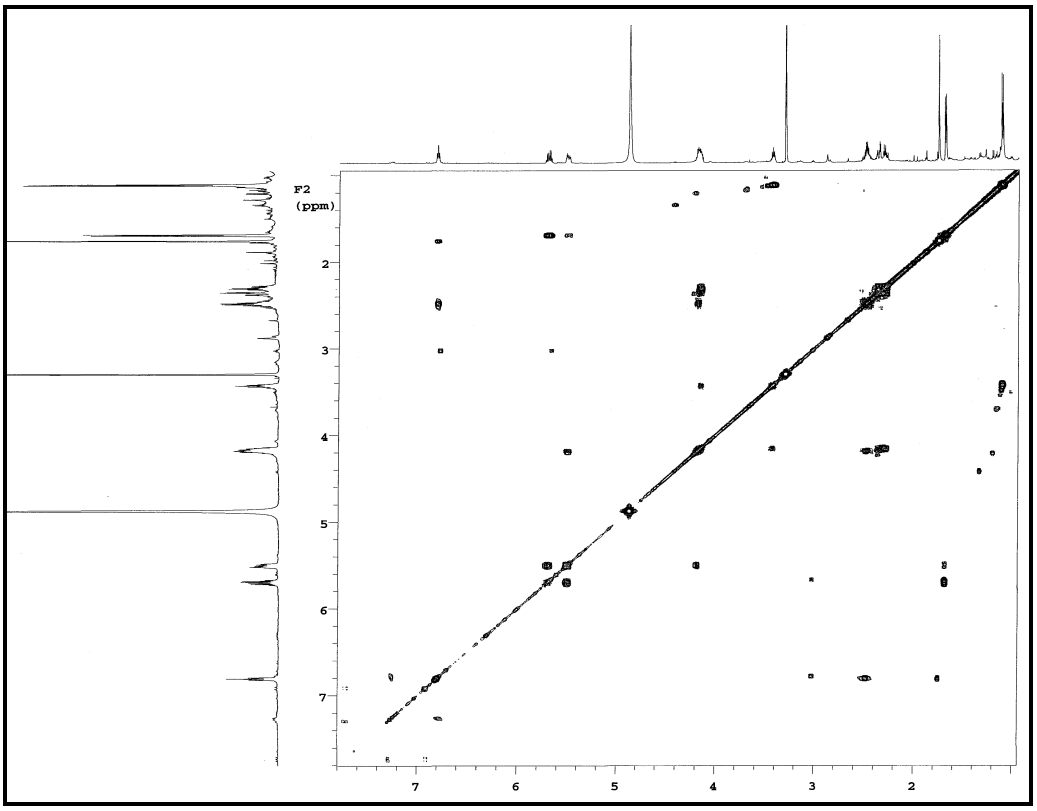
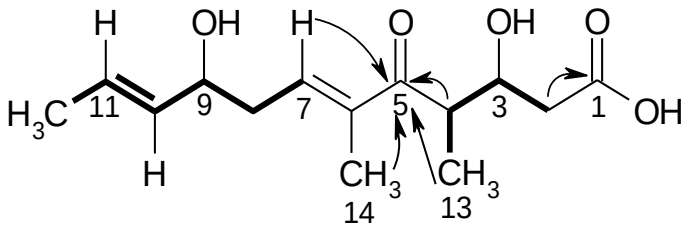


Figure 24: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of suhagcine I (**44**)

HMBC spectroscopic data confirmed the above fragments. The sp^2 bound methyl singlet at δ 1.76 (C-14, 11.9), the methyl doublet at δ 1.11 (C-13, 14.1), a methine multiplet at δ 3.43 (C-4, 45.7) and sp^2 a methine multiplet at δ 6.81 (C-7, 141.2) showed correlations to the ketone carbonyl at δ 206.4 (C-5). Furthermore, the acid carbonyl at δ 175.2 (C-1) showed correlation to the methylene multiplet at δ 2.33 (C-2, 41.1). The coupling constant between H-10 and H-11 was larger than 12 Hz and pointed to a *trans* double bond. According to these spectroscopic data, the following structure can be completed.



44

Figure 25: $^{1}H,^{1}H$ COSY (▬) and selected HMBC (→) correlations of suhagcine I (**44**)

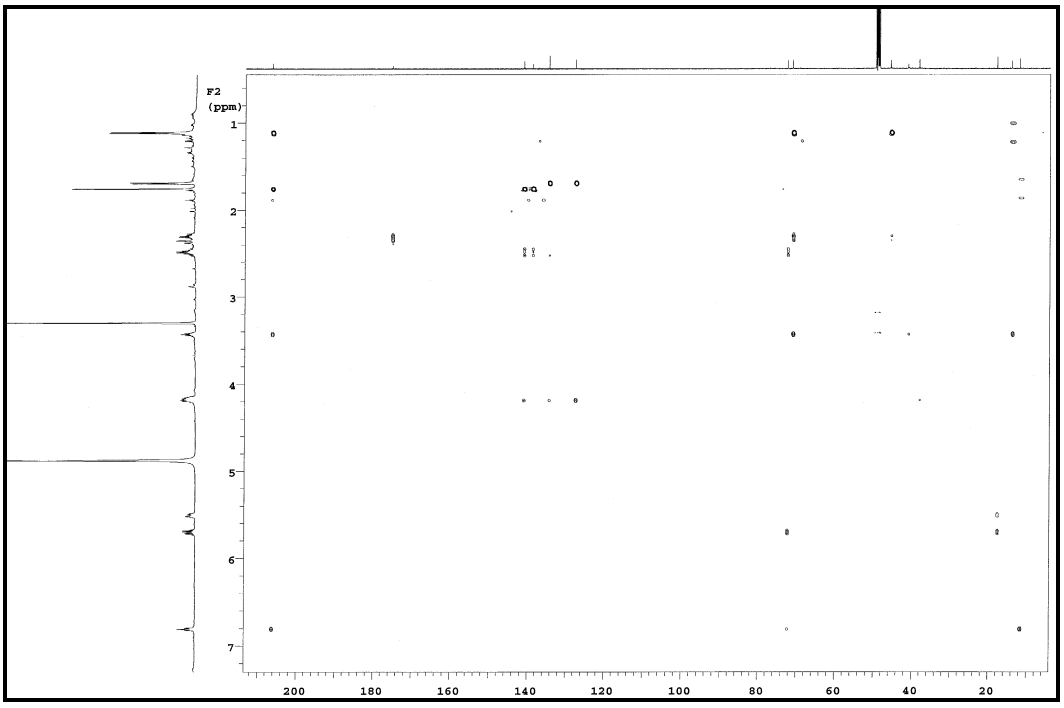


Figure 26: HMBC spectrum (CD_3OD, 500 MHz) of suhagcine I (**44**)

Table 5: ^{1}H and ^{13}C NMR spectroscopic data for suhagcine I (**44**)

Suhagcine I (**44**)				
Position	δ_C [a, b]	mult.	δ_H [a, c], mult. (*J* in [Hz])	HMBC
1	175.2	C_q	-	-
2	41.1	CH_2	2.33, m	1, 3, 4
3	71.1	CH	4.16, m	-
4	45.7	CH	3.43, m	2, 3, 5, 13
5	206.5	C_q	-	-
6	138.9	C_q	-	-
7	141.2	CH	6.81, t (7.0)	5, 14, 9
8	38.2	CH_2	2.48, m	6, 7, 9
9	72.5	CH	4.18, m	7, 8, 10, 11
10	134.6	CH	5.50, dd (16.1, 6.8)	12
11	127.6	CH	5.69, dq (16.1, 6.5)	9, 12
12	17.9	CH_3	1.69, d (6.4)	10, 11
13	14.1	CH_3	1.11, d (6.8)	3, 4, 5
14	11.9	CH_3	1.76, s	5, 6, 7

[a] CD_3OD; [b] 125 MHz; [c] 300 MHz

4.1.7 Suhagcine II

Suhagcine II (**45**) was isolated as colourless solid, which showed a UV absorbing band at 254 nm and was visualized on TLC plate by a blue colour on spraying with anisaldehyde-sulphuric acid. The ^{1}H NMR spectrum of suhagcine II (**45**) was similar to that of suhagcine I (**44**). By HRESIMS, the molecular formula was deduced as $C_{13}H_{20}O_4$. The ^{1}H NMR spectrum revealed three olefinic protons at δ 7.29 (H-6), 6.79 (H-7) and 6.39 (H-8) and three methine protons at δ 4.55 (H-3), 4.09 (H-9) and 3.71 (H-10), which should be connected with oxygen, according to their shifts. Furthermore, two methyl singlets were observed at δ 2.09 (H-1) and 1.89 (H-13); they could belong to acetyl groups or were connected with sp^2 carbon. Finally, two methyl doublets were found at δ 1.24 (H-12) and 1.18 (H-11).

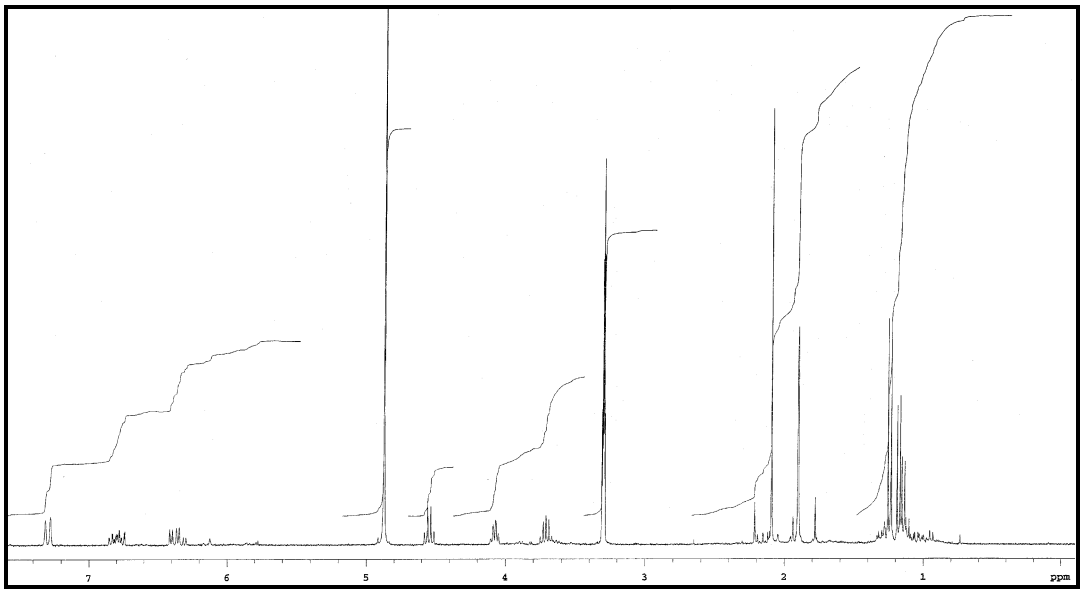


Figure 27: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of suhagcine (II) (**45**)

The ^{13}C NMR spectrum of **45** exhibited 13 carbon signals comprising of two ketone carbonyls at δ 207.4 and 201.2, four sp^2 carbon signals (three methine and one quaternary), three sp^3 methine signals and four methyl signals. According to the HSQC spectrum, the two methyl singlets at δ 2.09 (H-1) and 1.89 (H-13) were connected to carbons at δ 28.4 and 11.9, respectively. Due to their chemical shift, the former was assigned as an acetyl group and the latter was an sp^2-bound methyl. Two of three methine protons at δ 4.09 and 3.71 were assigned to oxymethine groups according to their chemical shifts.

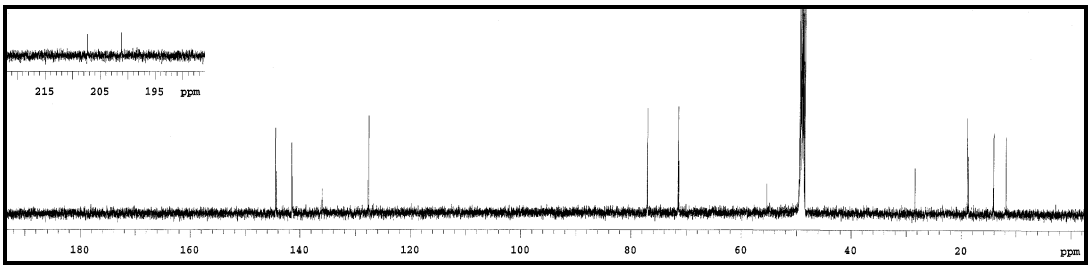


Figure 28: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of suhagcine II (**45**)

In the COSY spectrum, couplings from H-7 to H-6 and H-8, H-6 to H-13, H-8 to H-9, H-9 to H-10, H-10 to H-11 and H-3 to H-12 were observed. From these data, the following two fragments could be drawn. The coupling constant between the olefinic protons H-7 and H-8 was 14.8 Hz and pointed to their *trans* position.

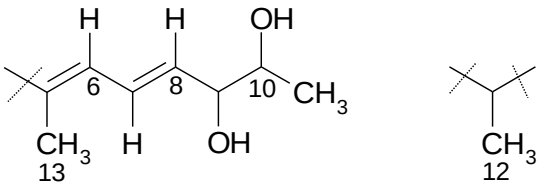


Figure 29: Selected $^{1}H,^{1}H$ COSY (▬) correlations of substructures

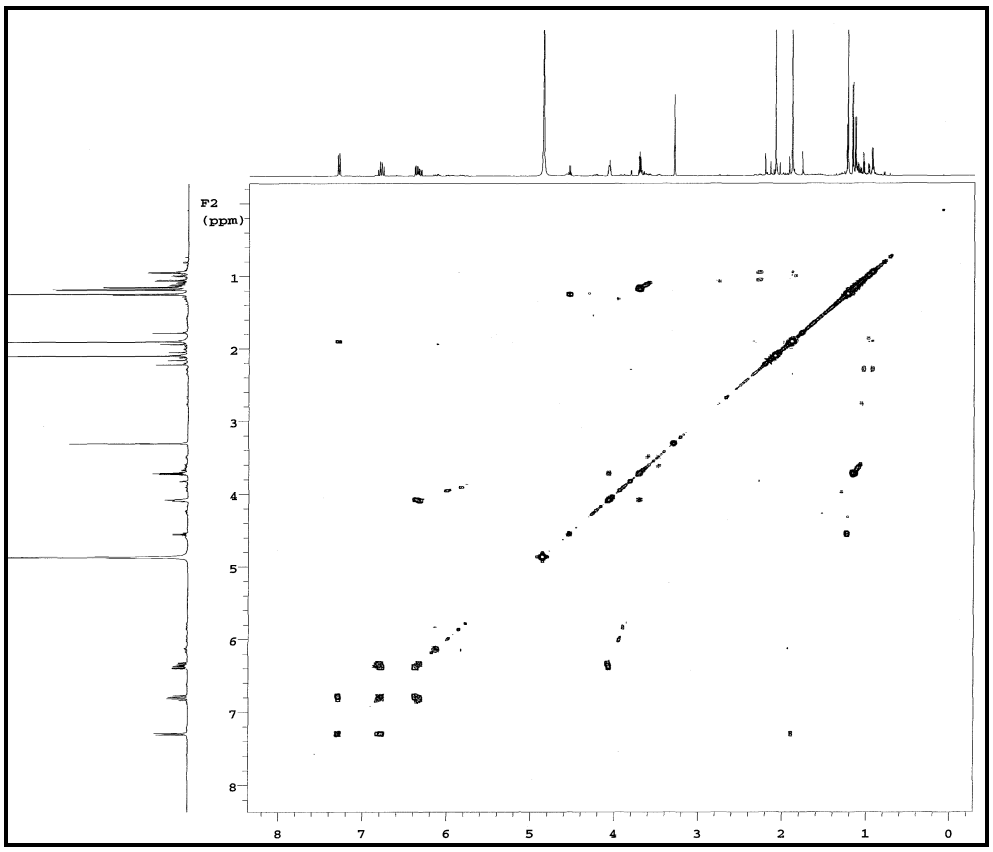


Figure 30: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of suhagcine II (**45**)

The HMBC spectrum confirmed the above correlations. Moreover, the ketone carbonyl at δ 201.2 (C-4) correlated with two methyl groups at δ 1.24 (H-12) and 1.89 (H-13). Therefore, these two fragments were connected with the carbonyl group. The both methyl protons H-12 and H-1 (δ 2.09), which is assigned to an acetyl group, both were correlated with the ketone carbonyl at δ 207.4 (C-2). Thus, the complete structure **45** can be deduced.

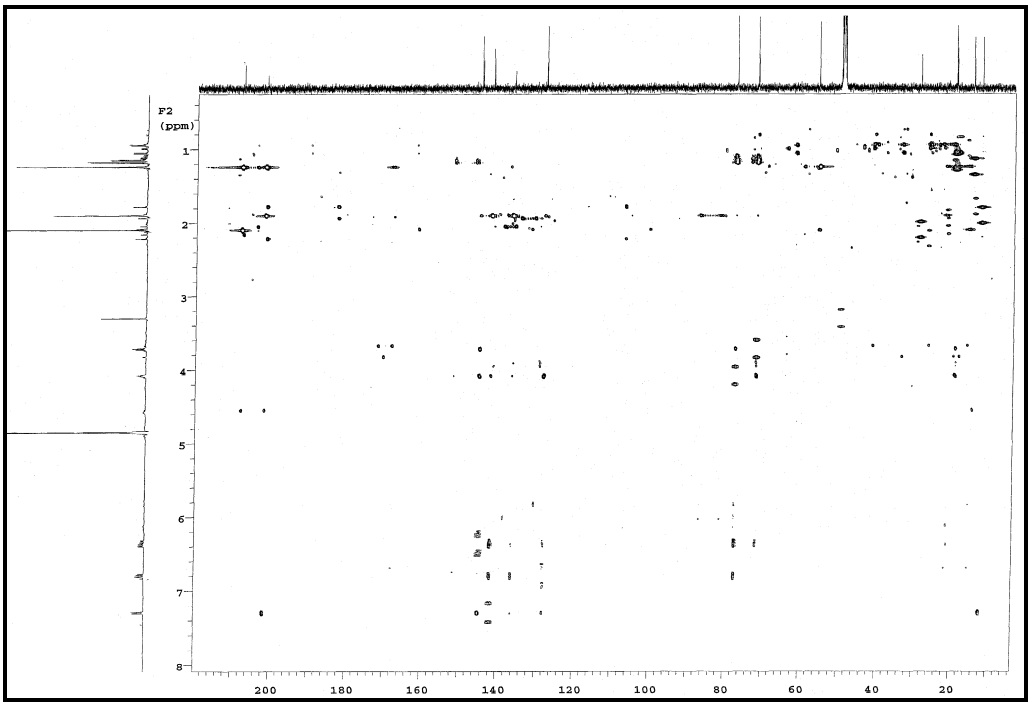
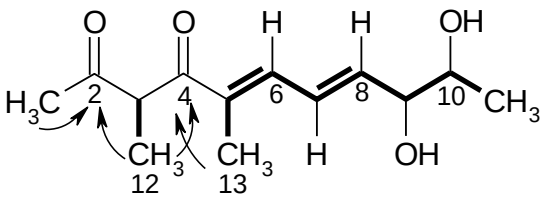


Figure 31: HMBC spectrum (CD_3OD, 600 MHz) of suhagcine II (**45**)



45

Figure 32: Selected HMBC (→) and $^1H,^1H$COSY (▬) correlations in suhagcine II (**45**)

Suhagcine I (**44**) and II (**45**) are structurally related to mikamycinine, which is a main hydrolysis product of mikamycin A[87] and mycinonic acids, which were proposed to be biosynthesis intermediates of the mycinamicin macrolide antibiotics. [88]

Table 6: ^{1}H and ^{13}C NMR data for suhagcine II (**45**)

suhagcine II (**45**)			
Position	δ_C [a, b], mult	δ_H [a, c], mult (*J* in [Hz])	HMBC
1	28.4, CH_3	2.09, s	2, 3, 12
2	207.4, C_q	-	
3	55.4, CH	4.55, q (7.0)	2, 4, 12
4	201.2, C_q	-	
5	136.0, C_q	-	
6	141.5, CH	7.29, d (11.2)	4, 7, 8, 13
7	127.7, CH	6.79, m	5, 6, 9
8	144.5, CH	6.39, dd (5.7, 14.8)	6, 9, 10
9	77.1, CH	4.09, q (5.6)	6, 7, 8, 10, 11
10	71.5, CH	3.71, m	8, 9, 11
11	19.0, CH_3	1.18, d (6.4)	8, 9, 10
12	14.2, CH_3	1.24, d (6.9)	2, 3, 4
13	11.9, CH_3	1.89, s	4, 5, 6

[a] CD_3OD; [b] (125 MHz); [c] (300 MHz)

4.1.8 2,5-Furandimethanol

2,5-Furandimethanol (**46**) isolated as oily substance from a UV absorbing zone in fraction III; it turned to dark brown with anisaldehyde/sulphuric acid. EI mass spectroscopy displayed the molecular weight as 128 Dalton. The ^{1}H NMR spectrum of 2,5-furandimethanol (**46**) exhibited in the olefinic region one 2H singlet at δ 6.22. Furthermore, two methylene groups gave a singlet at δ 4.47.

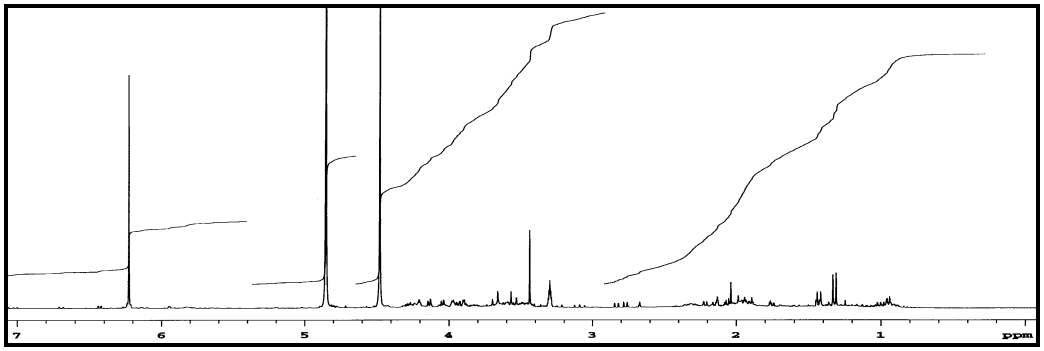
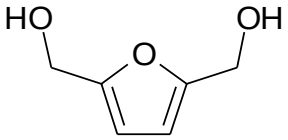


Figure 33: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 2,5-furandimethanol (**46**)

A search in AntiBase supported by ^{1}H NMR data led to 2,5-furandimethanol (**46**), which was further confirmed by comparison with literature data.[89] 2,5-Furandimethanol (**46**) displayed potent antifungal activities towards *Nematospora*

coryli and *Saccharomyces cerevisiae*. It was previously isolated from the culture fluids of a wood-inhabiting ascomycete.[90]



46

4.2 Terrestrial *Streptomyces* sp. ANK 251

The crude extract of the terrestrial *Streptomyces* sp. ANK 251 showed moderate antimicrobial activity against the tested microorganisms Figure 249 while the TLC analysis exhibited yellow, brown and blue coloured zones with anisaldehyde/sulphuric acid.

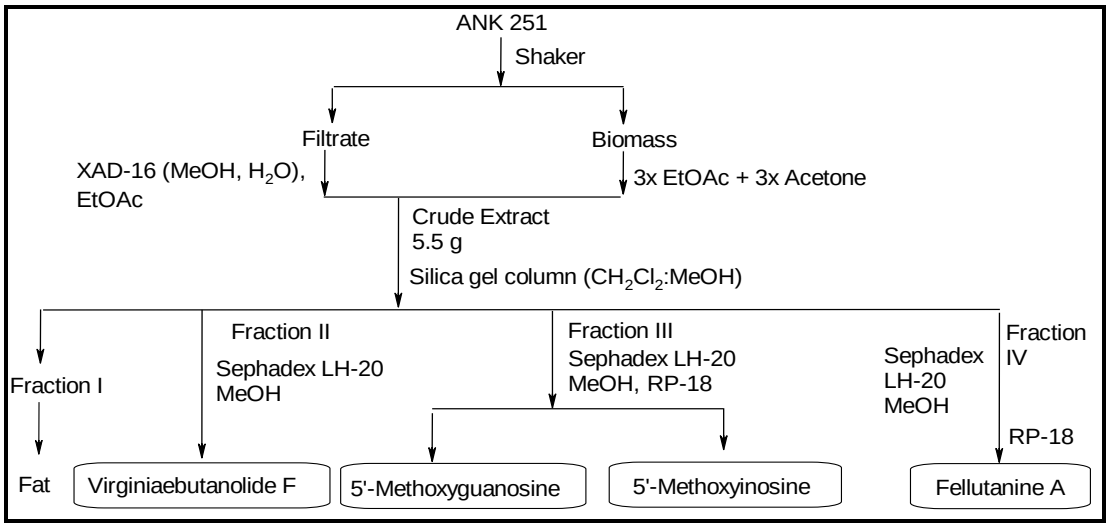


Figure 34: Work-up scheme of the terrestrial *Streptomyces* sp. ANK 251

4.2.1 Virginaebutanolide F

Virginaebutanolide F (**47**) was isolated as colourless solid from fraction II, which turned to blue with anisaldehyde reagent. The molecular weight of **47** was deduced from the ESI mass spectrum, which showed *pseudo*molecular ions at *m/z* 187 $[M-H]^-$, 375 $[2M-H]^-$, and 211 $[M+Na]^+$, 399 $[2M+Na]^+$, corresponding to a molecular weight of 188 Dalton. HRESIMS established the empirical molecular formula as $C_9H_{16}NaO_4$. The ^1H NMR spectrum of **47** displayed methylene protons at δ 4.42 and 4.08 as ABX system; their downfield shift was interpreted by an attachment to an sp^2

carbon or a heteroatom. Another methylene group was observed as doublet at δ 3.60, a methine proton appeared at δ 2.66. In addition to three methine groups were visualized; the first one was oxygenated and gave a multiplet at δ 3.30; the remaining methine groups were displayed at δ 2.75 and 2.14. Finally two methyl doublets were centred at δ 0.99.

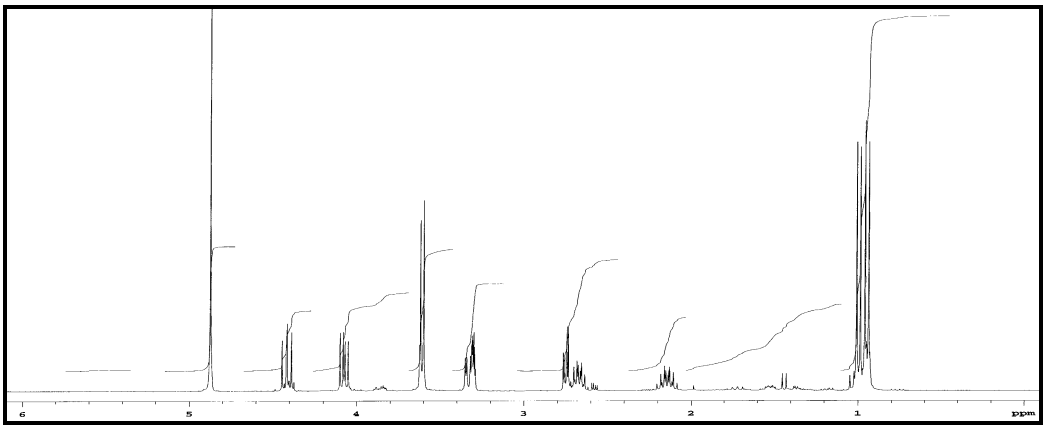


Figure 35: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of virginiabutanolide F (**47**)

The ^{13}C NMR and HSQC spectra showed 9 carbon signals, among them the carbonyl of an acid or ester at δ 179.3, an oxymethine at δ 78.8 and two methylene carbons at δ 70.9 and 63.3. In addition, two methine carbon signals at δ 46.5, 43.0 were observed. Finally, an isopropyl unit appeared at δ 32.3, 20.0 and 19.9.

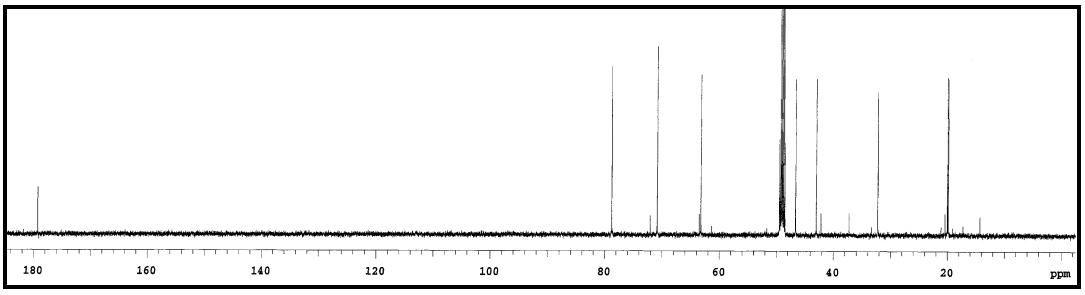


Figure 36: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of virginiabutanolide F (**47**)

The ^{1}H,^{1}H COSY spectrum showed strong correlation from a methine proton at δ 2.75 to other methine protons at δ 2.66 and at δ 3.30. The latter proton also showed COSY coupling with a proton at δ 2.14, which in turn showed correlations with the methyl at δ 0.99, hence confirming the presence of an isopropyl group. In addition, the methine at δ 2.66 correlated with methylene protons at δ_H 4.42, 4.08 (H-4) and showed strong coupling to another methylene at δ_H 3.60 (3-CH_2). The connectivity of the structure was confirmed by HMBC correlations, where three methine protons at δ

3.30, 2.75 and 2.66 and methylene protons at δ_H 4.42, 4.08 (H-4) showed ^3J couplings to the ester carbonyl at δ 179.3.

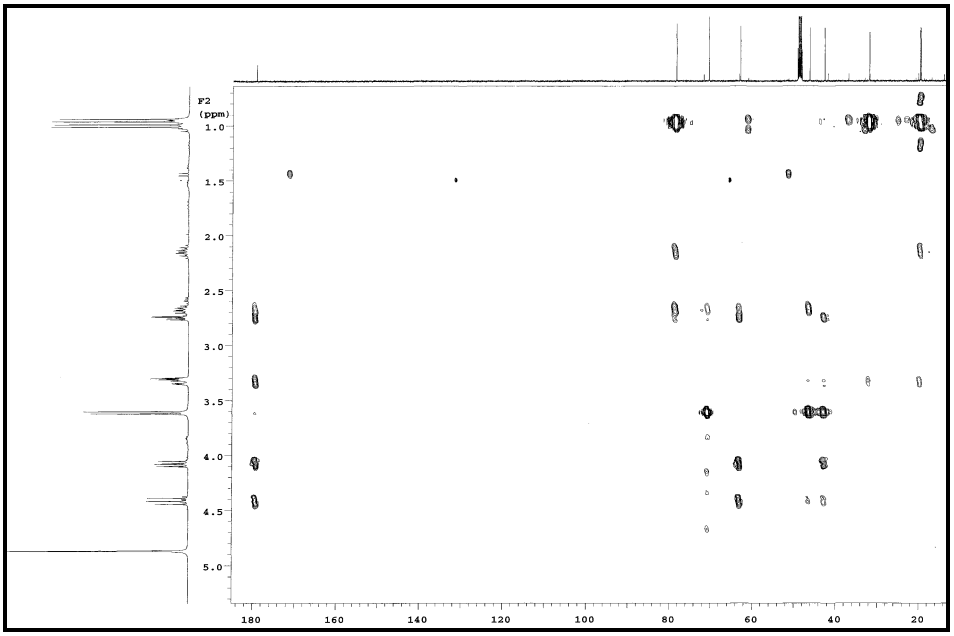


Figure 37: HMBC spectrum (CD_3OD, 600 MHz) of virginiabutanolide F (**47**)

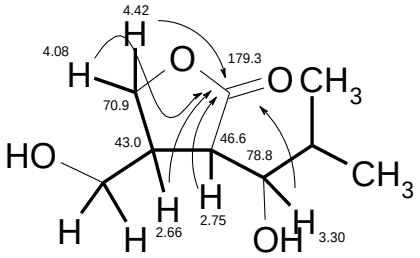


Figure 38: $^1H,^1H$ COSY (▬) and HMBC (→) of virginiabutanolide F (**47**)

Table 7: ^{13}C and ^1H NMR CD_3OD (125, 300 MHz) of virginiabutanolide F (**47**)

No.	δ_C, mult.	δ_H (mult.; J in [Hz])
1	179.3, C_q	-
2	46.6, CH	2.75 (dd, 6.2, 2.8)
3	43.0, CH	2.66 (m)
3-CH_2	63.3, CH_2	3.60 (d, 5.8)
4	70.9, CH_2	4.42 (ABX, 8.9, 8.0)
		4.08 (ABX, 8.9, 5.6)
1'	78.8, CH	3.30 (m)
2'	32.3, CH	2.14 (m)
3'-CH_3	20.0, CH_3	0.99 (d, 6.6)
3'-CH_3	19.9 CH_3	0.99 (d, 6.6)

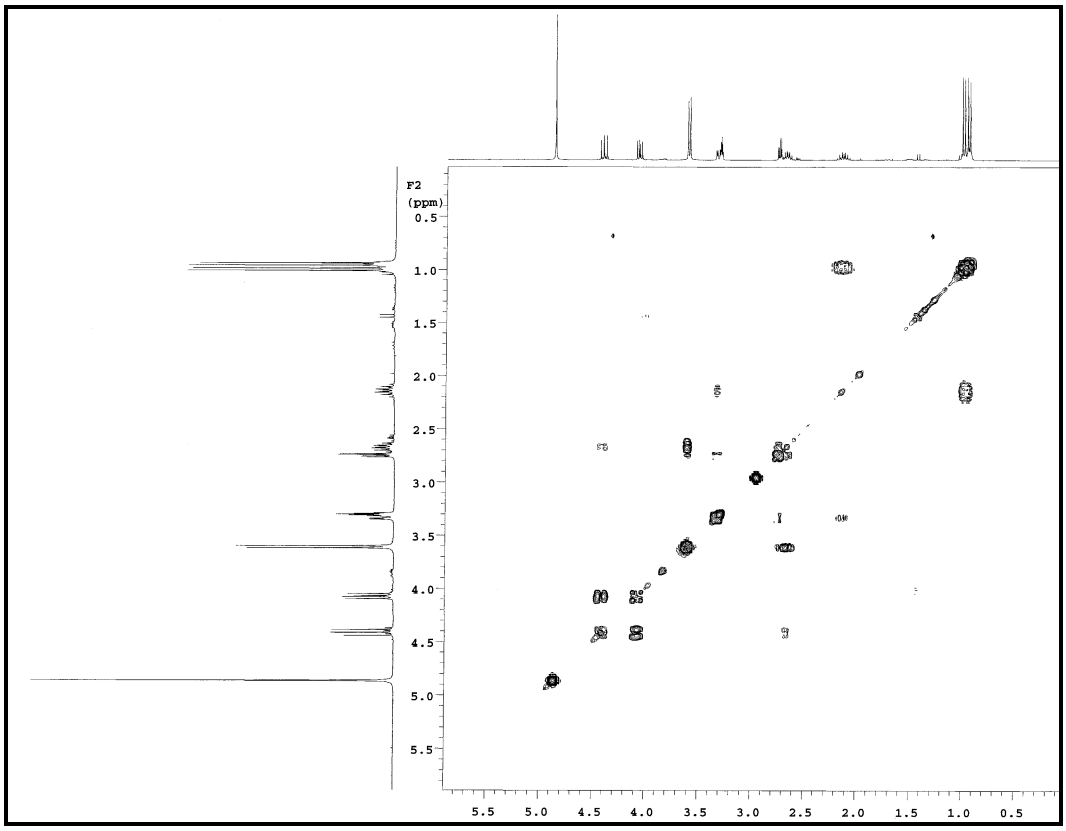
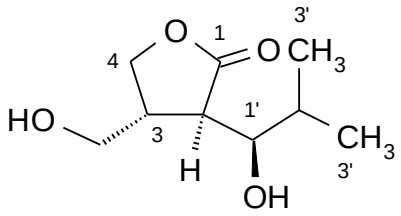


Figure 39: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of virginiabutanolide F (**47**)



47

A search in AntiBase[77] on the basis of extensive 1D and 2D NMR date as well as comparison with literature values led to virginiaebutanolide F (**47**), which was already isolated before from *Streptomyces* sp.[91]

4.2.2 5'-Methoxyinosine

5'-Methoxyinosine (**48**) was isolated as colourless solid from fraction III, which exhibited a brownish colour with anisaldehyde/sulphuric acid. ESIMS of compound **48** indicated a molecular weight of 282 Dalton, and HRESIMS confirmed the molecular formula as $C_{11}H_{15}N_5O_5$. The ^{1}H NMR spectrum exhibited two singlets at δ 8.30

and 8.05 as a hint for a purine derivative. In addition, an anomeric proton appeared at δ 6.05 as doublet. The spectrum further showed three oxymethine signals at δ 4.54 (H-2'), 4.31 (H-3'), 4.17 (H-4) and methylene protons at δ 3.71 and 3.62 as ABX system. Finally a 3H singlet at δ 3.42 suggested the presence of a methoxy group.

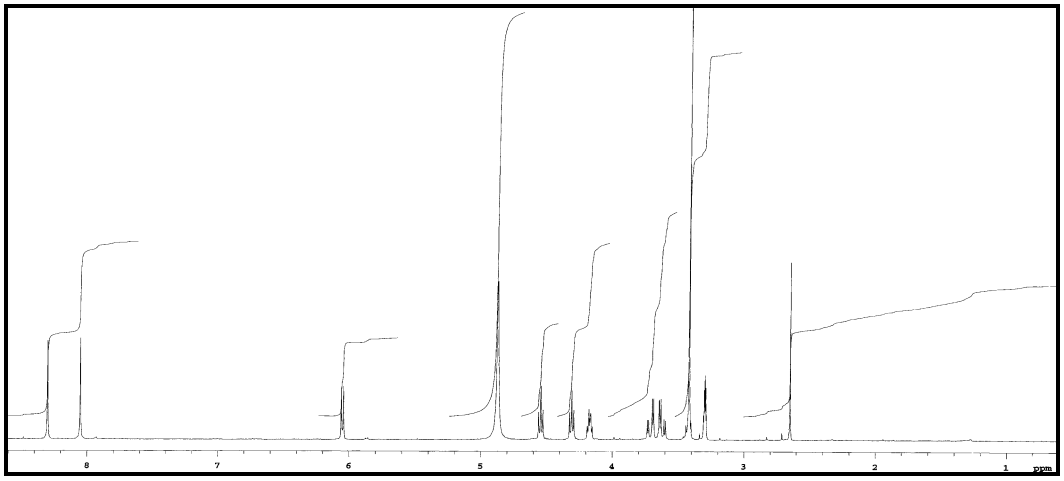


Figure 40: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 5'-methoxyinosine (**48**)

The ^{13}C NMR and HSQC spectra showed eleven carbon signals as established from HRESIMS. Among them, three quaternary sp^2 carbons at δ 158.8 (C-4), 149.9 (C-7a) attached to heteroatoms and third quaternary carbon at δ 125.4 (C-4a) and two methine carbons at δ 146.7 (C-2), 140.1 (C-6) were observed. Additionally, an *O*-methylated β-ribosyl moiety was identified by a signal at δ 59.9.

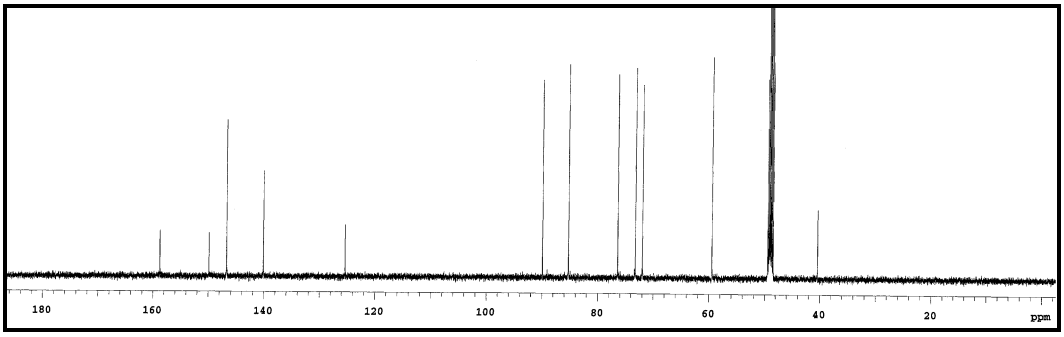


Figure 41: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 5'-methoxyinosine (**48**)

Applying the above data to a search in AntiBase resulted in no hits, pointing to a new natural product. So, the structure was further analysed by 2D correlations. In the ^{1}H,^{1}H COSY spectrum, ^{3}J correlation from the anomeric proton at δ 6.05 to a triplet at δ 4.54 were displayed. The latter showed strong coupling to another triplet at δ 4.41. Methylene protons at δ H_2-5' (H 3.71/3.62) exhibited ^{3}J correlation to a quartet

at δ_H 4.17, confirming their direct linkage. All these correlations confirmed the presence of furanosyl moiety (fragment **A**)

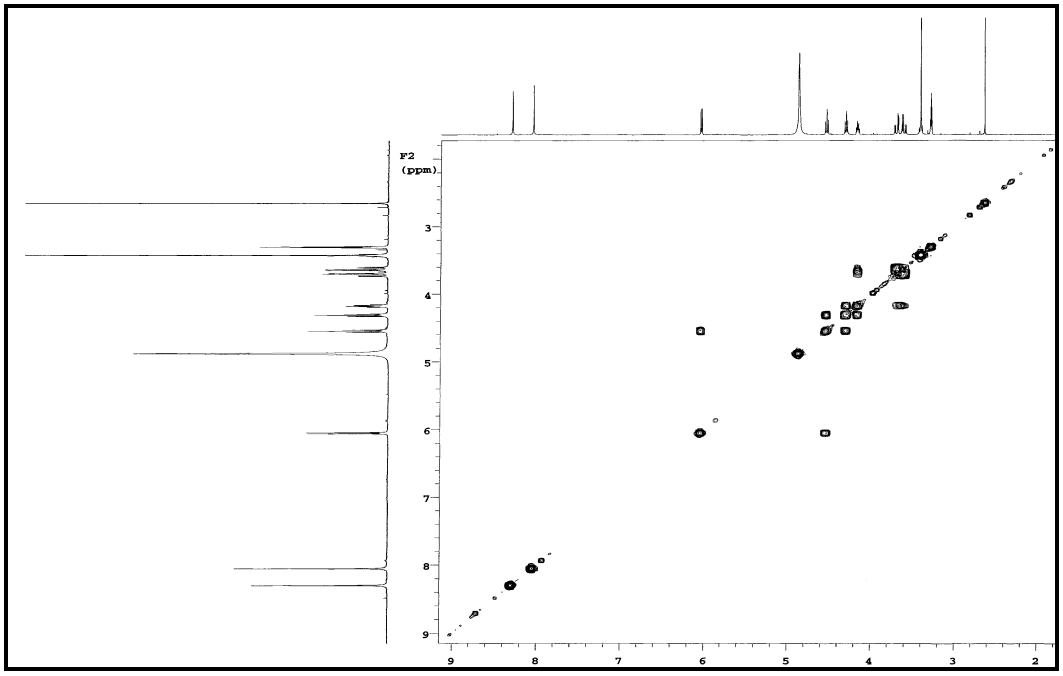
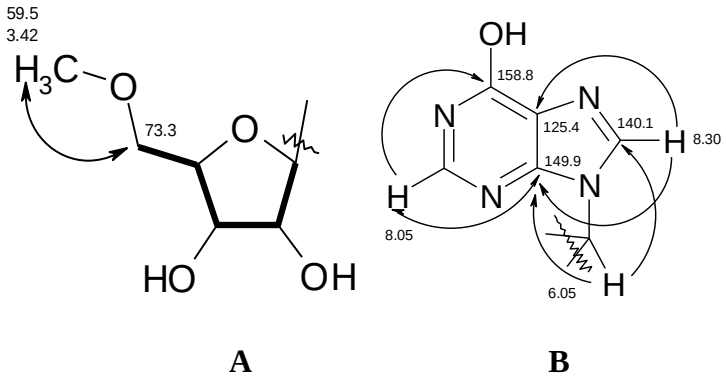


Figure 42: ^{1}H,^{1}H COSY spectrum (CD_3OD, 600 MHz) of 5'-methoxyinosine (**48**)

Based on HMBC connectivities, both the methoxy group and the oxymethine proton at H-3' (δ 4.31) exhibited ^3J cross-signals with the methylene carbon at C-5' (δ 73.3) to confirm that the methoxy group was attached directly to (C-5'). The purine fragment was established by HMBC correlations. The singlet at δ 8.05 (H-2) showed strong correlations to both quaternary carbons at δ 158.8 (C-4) and 125.4 (C-4a). The doublet at δ 6.05 of the anomeric proton exhibited ^3J cross-signals to both aromatic carbons at δ 140.1 (C-6) and the quaternary carbon at δ 149.9 (C-7a) to explain the attachment of the sugar moiety with N-7 (fragment **B**).



A **B**

Figure 43: ^{1}H-^{1}H COSY (▬) and HMBC (→) couplings of 5'-methoxyinosine (**48**)

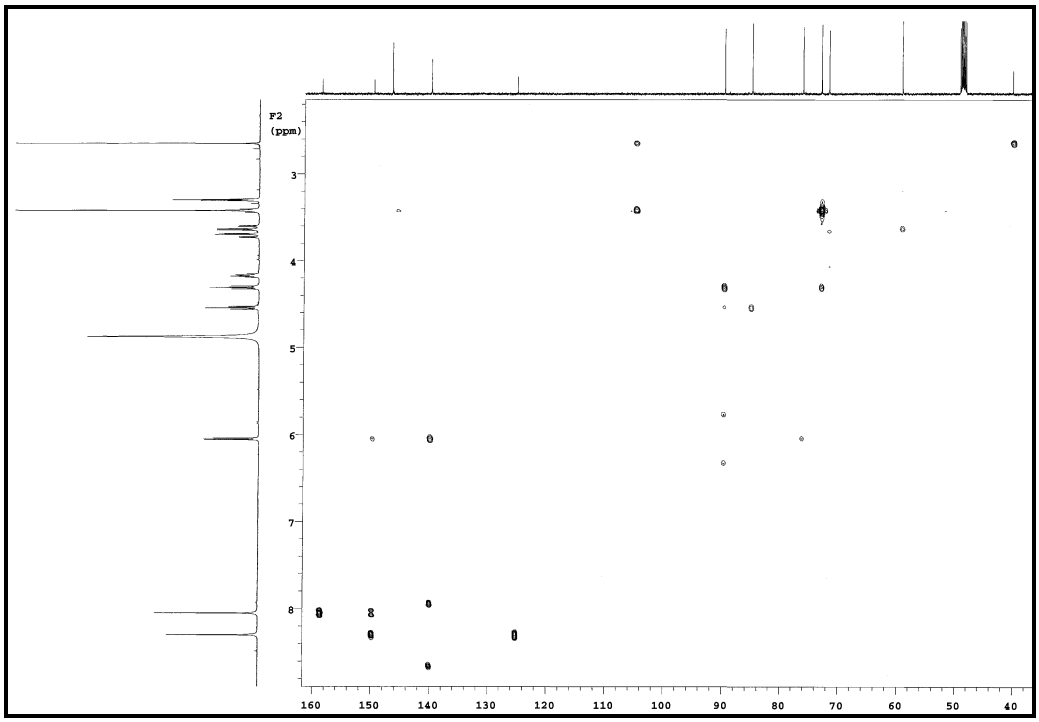
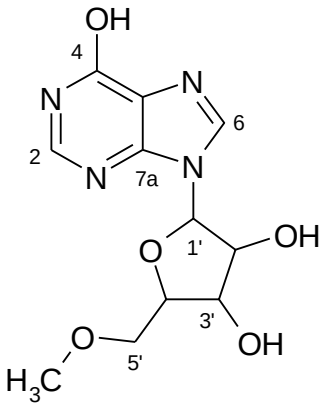


Figure 44: HMBC spectrum (CD_3OD, 600 MHz) of 5'-methoxyinosine (**48**)



48

Table 8: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of 5'-methoxyinosine (**48**) in CD_3OD

No.	δ_c, mult.	δ_H (mult.; *J* in [Hz])
2	146.7, CH	8.05 (s)
4	158.8, C_q	-
4a	125.4, C_q	-
6	140.1, CH	8.30 (s)
7a	149.9, C_q	-
1'	90.0, CH	6.05 (d, 4.7)
2'	76.4, CH	4.54 (t, 4.9)
3'	72.0, CH	4.31 (t, 4.7)
4'	85.3, CH	4.17 (q, 3.5)
5'	73.3, CH_2	3.71 (dd, 10.8, 3.0)
		3.62 (dd, 10.8, 3.7)
5'-OCH_3	59.5, CH_3	3.42 (s)

4.2.3 5'-Methoxyguanosine

5'-Methoxyguanosine (**49**) was isolated from the same fraction III as colourless solid. The molecular weight of **49** was deduced as 297 Dalton from the *pseudo*molecular ions at *m/z* 296 $[M-H]^-$ and *m/z* 320 $[M+Na]^+$ (ESI MS). The odd mass number was an indication of an odd number of nitrogen atoms in the molecular formula. HRESIMS established the empirical molecular formula as $C_{11}H_{15}N_5O_5$. The ^{1}H NMR pattern displayed a very close similarity with that of 5'-methoxyinosine (**48**) except that two singlets in the aromatic region had disappeared and were replaced by a singlet at δ 7.74.

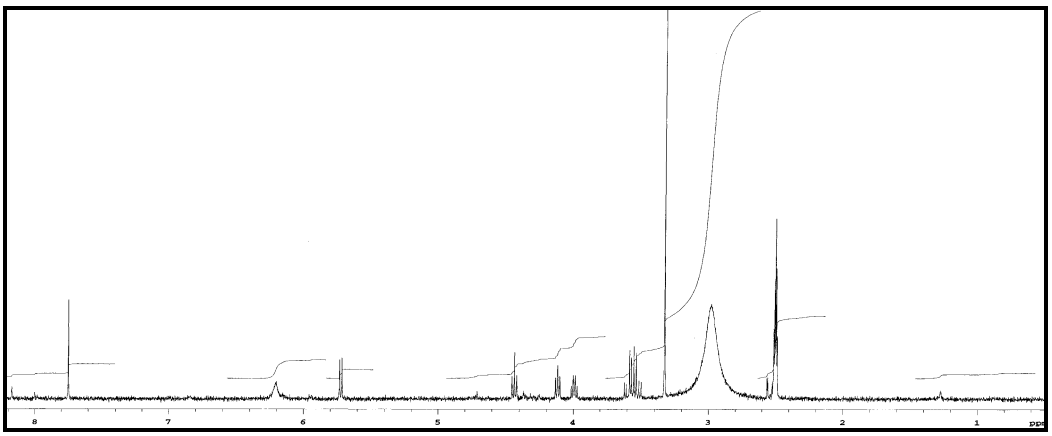


Figure 45: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of 5'-methoxyguanosine (**49**) (100 °C)

The ^{13}C NMR and HMQC spectra showed 11 carbon signals including three sp^2 carbons attached to heteroatoms at δ 156.8, 153.8 and 151.5. An sp^2 methine signal at δ 135.5 and a quaternary carbon at δ 116.7 . Additionally, an anomeric carbon signal appeared at δ 86.5. Three oxymethine signals were exhibited at δ 82.9, 73.3 and 70.5; in addition, an oxymethylene carbon was observed at δ 72.4, along with a methoxy carbon at δ 58.5.

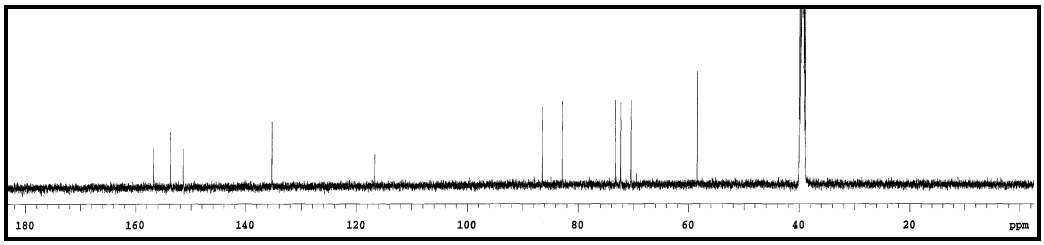


Figure 46: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of 5'-methoxyguanosine (**49**)

Based on COSY correlations, the anomeric proton at δ 5.72 (H-1') exhibited ^3J cross-signals with δ 4.44 (H-2'), which correlate to an oxymethine at δ 4.11. In addition, methylene protons at δ 3.59 and 3.52 adjacent to methine proton at δ 3.99 were observed (fragment **A**). The HMBC spectra displayed strong ^3J coupling of the methoxy protons at δ 3.32 with the methylene carbon at δ 72.4 *(*C-5'), and the anomeric proton exhibited strong correlations to a quaternary carbon at δ 151.5 and methine sp^2 carbon at δ 135.5, which itself saw two aromatic carbons at δ 116.7 and 151.5. (Fragment **B**)

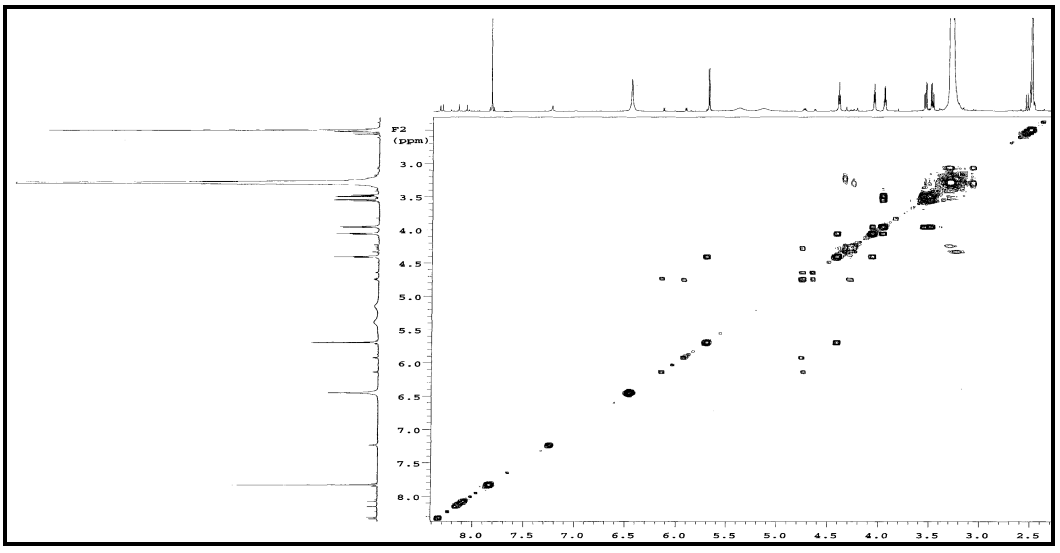
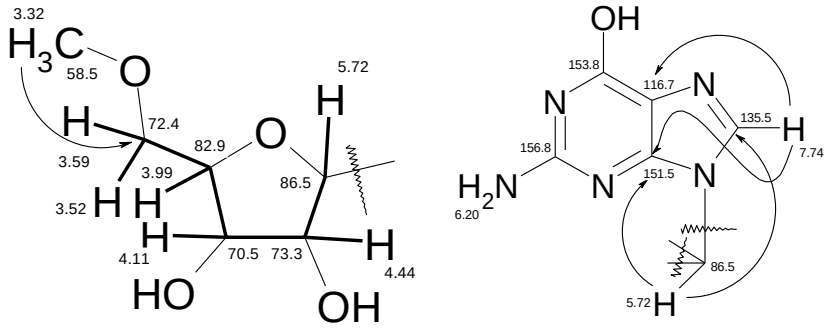


Figure 47: ^{1}H,^{1}H COSY spectrum (DMSO-d_6, 600 MHz) of 5'-methoxyguanosine (**49**)



A **B**

Figure 48: ^{1}H,^{1}H COSY (▬) and HMBC (→) correlations of 5'-methoxyguanosine (**49**)

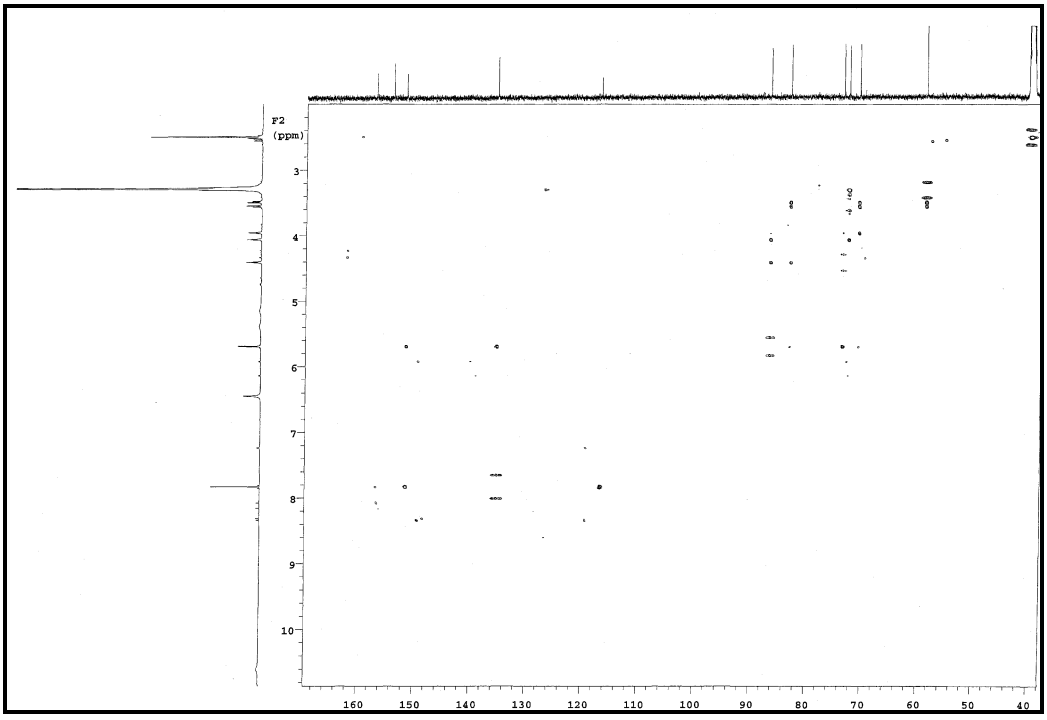
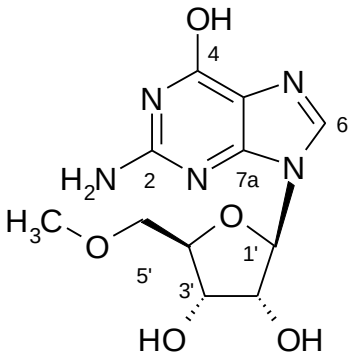


Figure 49: HMBC spectrum (DMSO-d_6, 600 MHz) of 5'-methoxyguanosine (**49**)

According to NOESY correlations of the proton at δ 4.44 (CH-2') with the aromatic proton at δ 7.74 and H-3' (4.11) and of a correlation of the anomeric proton at δ 5.72 with H-4' at δ 3.99, a *ribo*-configuration was assumed. According to the Klyne rule[92], the observed β-configuration fits best with D-ribose.



49

Table 9: ^{13}C and ^{1}H NMR data (125, 300 MHz) of 5'-methoxyguanosine (**49**) in DMSO-d_6 (35 °C)

No.	δc, mult.	δ_H (mult.; J in [Hz])
2	156.8, C_q	-
4	153.8, C_q	-
4a	116.7, C_q	-
6	135.5, CH	7.74 (s)
7a	151.5, C_q	-
1'	86.5, CH	5.72 (d, 5.2)
2'	73.3, CH	4.44 (t, 5.3)
3'	70.5, CH	4.11 (t, 4.9)
4'	82.9, CH	3.99 (q, 4.2)
5'	72.4, CH_2	3.59 (ABX, 11.0, 4.0)
		3.52 (ABX, 11.0, 5.0)
5'-OCH_3	58.5, CH_3	3.32 (s)
4-OH	-	10.60 (s br, 1H)
2-NH_2	-	6.42 (s br)
2'-OH	-	5.38 (d, 5.4, 1H)
3'-OH	-	5.14 (d, 4.4, 1H)

4.2.4 Fellutanine A

The molecular weight of **50** was deduced by ESIMS, which showed *pseudo*molecular ions at *m/z* 371 $[M-H]^-$, 743 $[2M-H]^-$, 395 $[M+Na]^+$, and 767 $[2M+Na]^+$, corresponding to a molecular weight of 372 Dalton. HRESIMS established the molecular formula as $C_{22}H_{20}N_6O_2$. The ^{1}H NMR spectrum of **50** exhibited in the aromatic region four proton signals, two were doublets centred at δ 7.44 (H-4) and 7.29 (H-7)

and two were doublets of doublets at δ 7.08 (H-6) and 7.00 (H-5). In addition, a singlet at δ 6.45 (H-8a) was observed. The chemical shifts and signal patterns indicated the presence of an indole moiety. In the aliphatic region an oxymethine at δ 4.04 and a methylene group appeared as ABX system at δ 2.91 and 2.16.

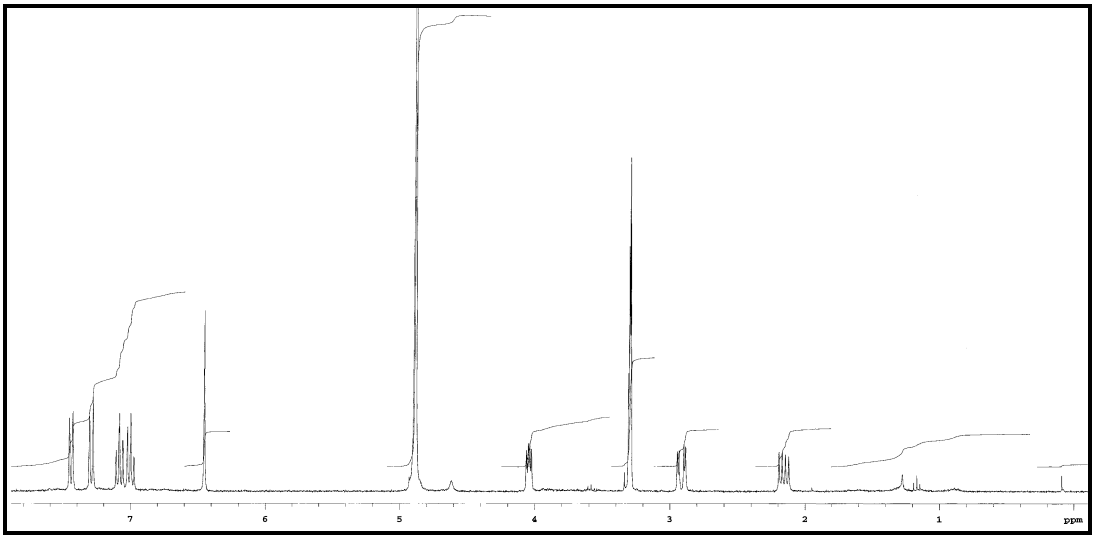


Figure 50: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of fellutanine A (**50**)

The ^{13}C NMR and HMQC spectra exhibited 11 carbon signals including a carboxamide at δ 169.6, eight sp^2 carbon signals, five methine carbons and three quaternary carbons. Moreover, a methine carbon attached to a heteroatom at δ 56.4 and a methylene carbon at δ 31.4 was also exhibited.

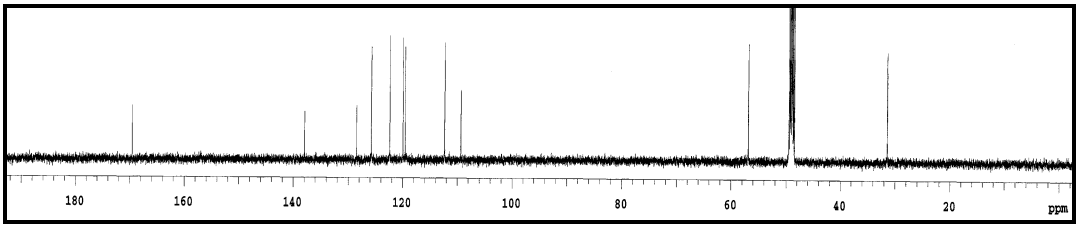


Figure 51: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of fellutanine A (**50**)

The presence of a benzene ring was confirmed by strong COSY correlations from the doublet at δ 7.44 to a proton at δ 7.00; the later exhibited ^{3}J correlations to a proton at δ 7.08, which in turn correlated to a proton at δ 7.29. The latter proton exhibited HMBC correlations with quaternary carbons at δ 137.9 and 128.5, which was confirmed by correlation from the proton at δ 119.6. On the other hand, proton H-8a (δ 6.45) showed ^{3}J coupling with the two quaternary carbons at δ 137.9, 128.5 and quaternary carbon at δ 109.4 to confirm the indole moiety. In addition, the methylene

protons at δ 31.4 showed three-bond correlations with the carboxamide at δ 169.6. Furthermore, correlations from the methine proton at δ 4.04 to the methylene protons at δ 2.16 and 2.91 were observed in the COSY spectrum.

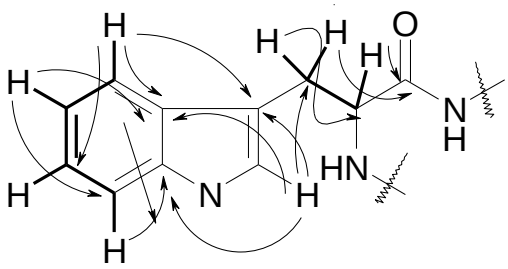


Figure 52: Selected ^{1}H,^{1}H COSY and HMBC correlations of fellutanine A (**50**)

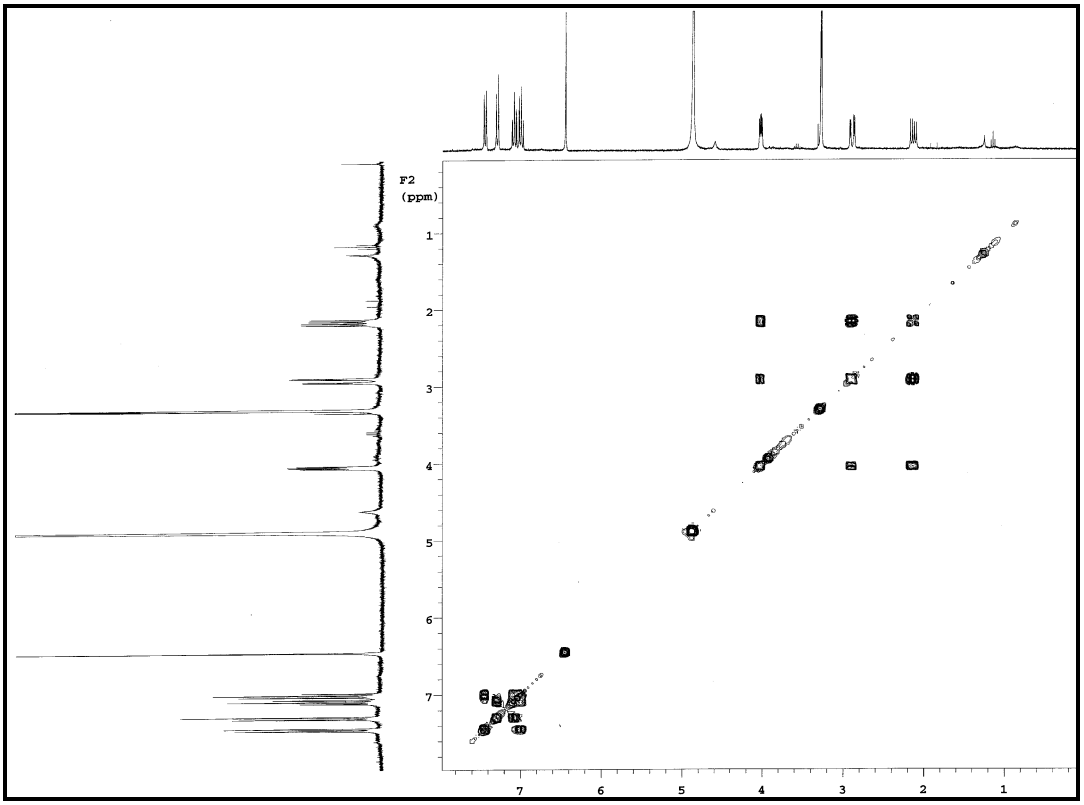
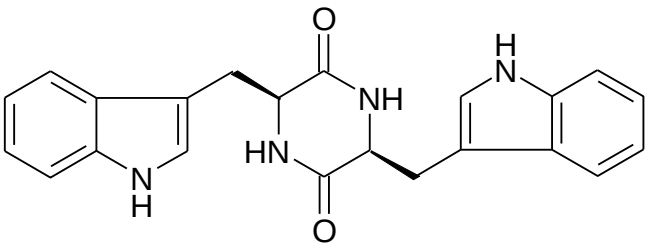


Figure 53: ^{1}H,^{1}H COSY spectrum (CD_3OD, 600 MHz) of fellutanine A (**50**)

A search in AntiBase and comparing the observed spectroscopic data with authentic spectra, confirmed the structure as the diketopiperazine fellutanine A (**50**).



50

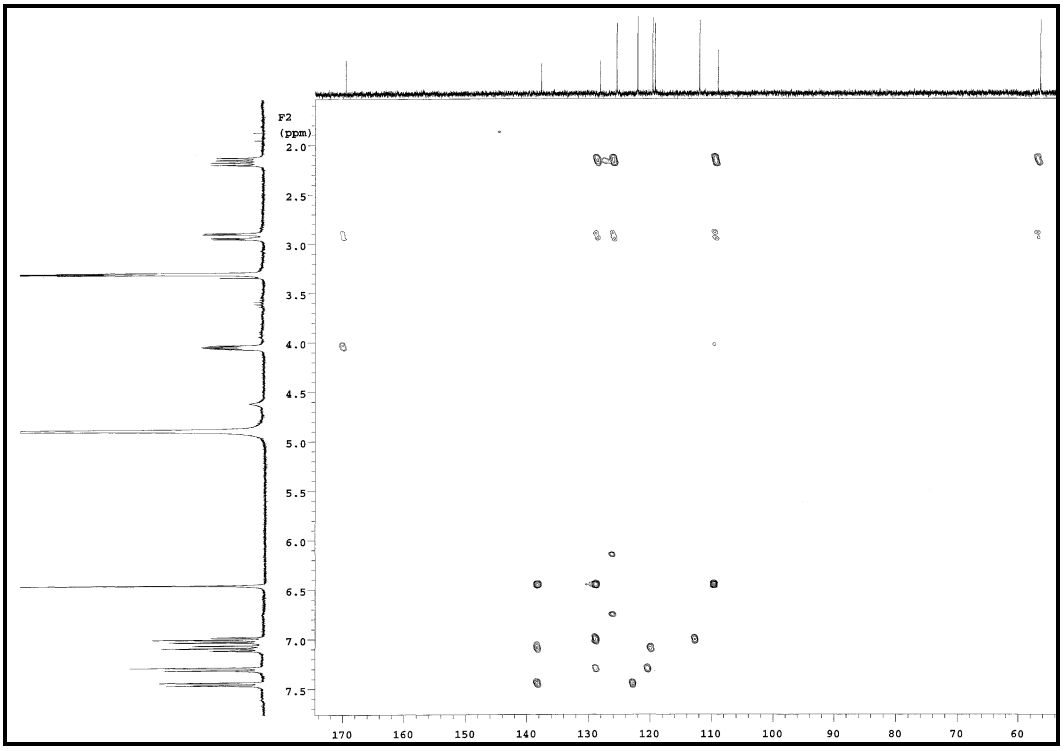


Figure 54: HMBC spectrum (CD_3OD, 600 MHz) of fellutanine A (**50**)

Fellutanine A (**50**) was isolated for first time from *Penicillium fellutanum*, [93] *Penicillium fungi*; [94] fellutanine B was also isolated from fungi of the genus *Penicillium.*[95]

Table 10: ^{13}C and ^{1}H NMR shifts (125, 300 MHz) of fellutanine A (**50**) in CD_3OD

No.	*δc*, mult.	δ_H (mult.; *J* in [Hz])
2	56.9, CH	4.04 (dd, 6.9, 3.8)
3	31.4, CH_2	2.16 (dd, 14.2, 7.3)
		2.91 (dd, 14.2, 3.5)
3a	109.4, C_q	-
3b	128.5, C_q	-
4	119.6, CH	7.44 (d, 7.8)
5	120.0, CH	7.00 (dd, 8.0, 1.1)
6	122.4, CH	7.08 (dd, 7.1, 1.1)
7	112.3, CH	7.29 (d, 8.0)
7a	137.9, C_q	-
8a	125.8, CH	6.45 (s)
9	169.6, C_q	-

4.3 Terrestrial *Streptomyces* sp. ANK 275

The terrestrial *Streptomyces* sp. ANK 275 was selected according to the chemical and biological screening. The crude extract showed good biological activities against different microorganisms as mentioned in Figure 250.

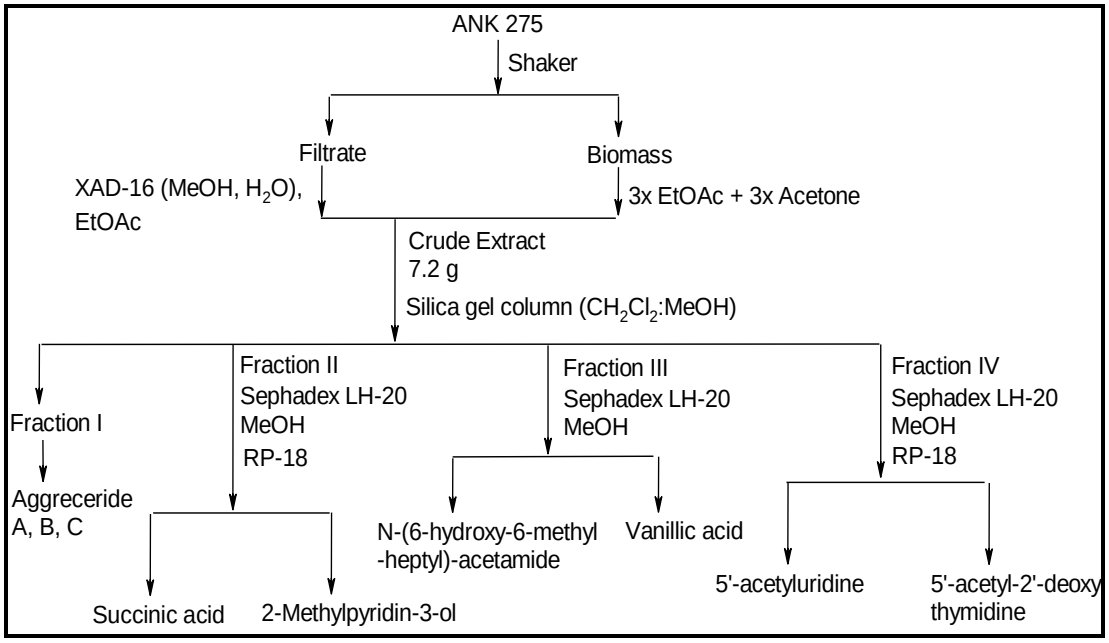


Figure 55: Work-up scheme terrestrial *Streptomyces* sp. ANK 275.

4.3.1 Succinic acid

Succinic acid (**51**) was isolated as colourless and UV inactive solid, which showed no colour with anisaldehyde reagent. The ESI mass spectrum of **51** displayed $[2M+Na]^+$ and $[M+Na]^+$ ion peaks at *m/z* 259 and 141 in positive mode, respectively. The (-)-ESI mass spectrum showed $[2M-H]^-$ and $[M-H]^-$ ion peaks at *m/z* 235 and 117, respectively, which fixed the mass as 118 Dalton. The high resolution MS determined the formula as $C_4H_6O_4$. In the ^1H NMR spectrum of **51** only one singlet at δ 2.55 in the aliphatic region was observed. A search in AntiBase supported by ^1H and MS spectroscopic data led to succinic acid (**51**) as a result. It was further confirmed by the comparison with authentic spectra.

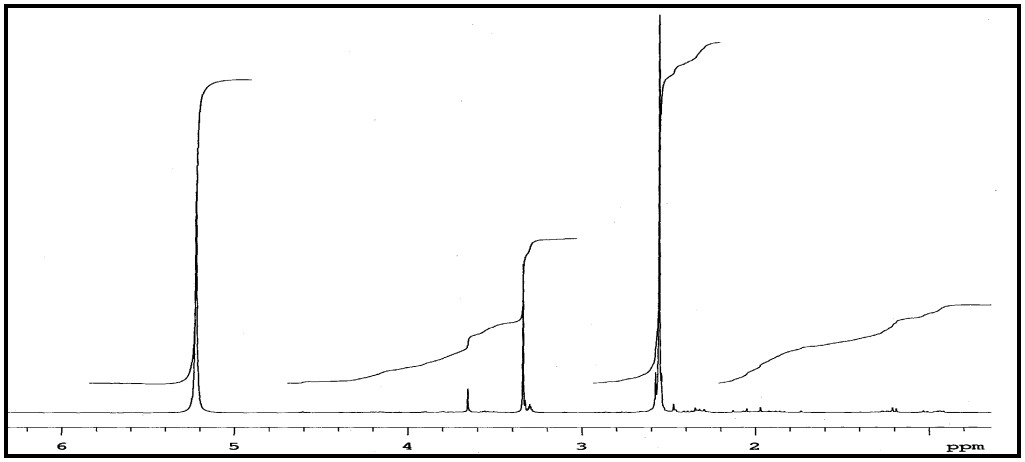
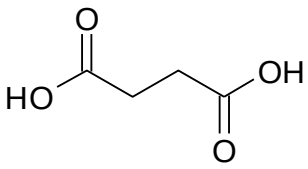


Figure 56: ^1H NMR spectrum (CD_3OD, 300 MHz) of succinic acid (**51**)

Barrero *et al*. isolated succinic acid (**51**) from the zygomycete *Phycomyces blakesleeanus.*[96]



51

4.3.2 2-Methylpyridin-3-ol

2-Methylpyridin-3-ol (**55**) was isolated as colourless powder. It appeared as dark spot on TLC under UV at 254 nm and changed to violet with anisaldehyde/sulphuric acid. ESIMS indicated a molecular weight of *m/z* 109, and HRESIMS confirmed the molecular formula as C_6H_7NO. The ^1H NMR spectrum of **55** revealed in the aromatic region three protons at δ 7.87 (d, 4.7 Hz), 7.20 (d, 8.2 Hz) and 7.14 (dd, 4.8, 8.1 Hz). The coupling pattern and the coupling constants indicated a 1,2,3-trisubstituted six remembered ring containing a heteroatom. Additionally, in the aliphatic region a methyl singlet at δ 2.41 was observed.

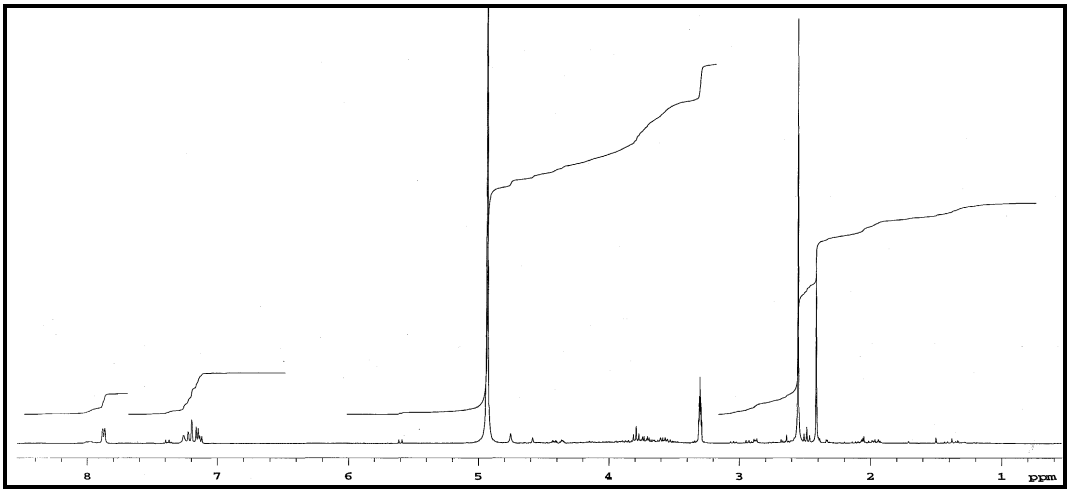


Figure 57: ^1H NMR spectrum (CD_3OD, 300 MHz) of 2-methylpyridin-3-ol (**55**)

The ^{13}C NMR and HMQC spectra revealed the presence of six carbon signals, among them two carbons at δ 154.1, 147.3, suggesting the connection to heteroatoms. Additionally, an aromatic methine at δ 138.6, connected to a heteroatom, two overlapped methine groups at δ 124.0 and a methyl carbon at δ 18.0 were observed.

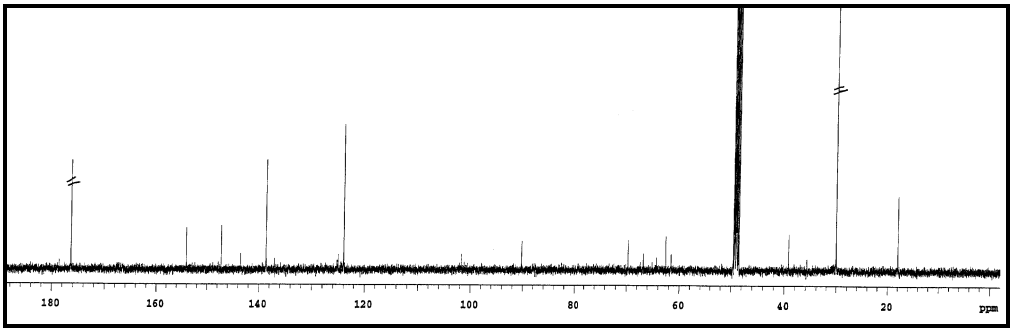


Figure 58: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 2-methylpyridin-3-ol (**55**)

The structure of **55** was confirmed by ^{1}H,^{1}H COSY and HMBC spectra: a proton at δ 7.87 showed COSY correlations with a proton at δ 7.14, and both revealed clear ^{3}J COSY correlations with a proton at δ 7.20. Beside HMBC correlations, the methyl signal at δ_H 2.41 showed strong correlation to quaternary carbons at δ_C 154.1 and 147.3. In addition, HMBC correlations were seen between a methin proton (δ_H 7.87) and both the quaternary carbon at δ_C 154.1 and the methine carbon (δ_C 124.0).

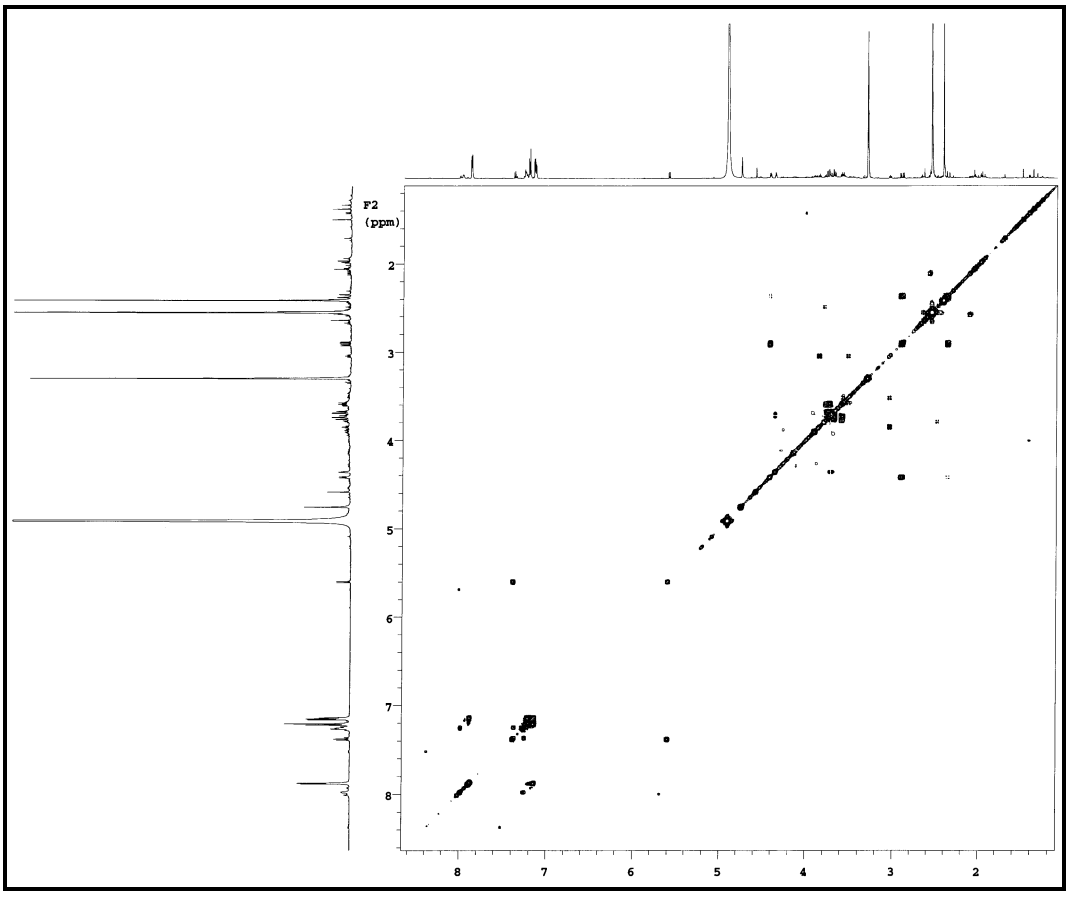


Figure 59: ^{1}H,^{1}H COSY spectrum (CD_3OD, 125 MHz) of 2-methylpyridin-3-ol (**55**)

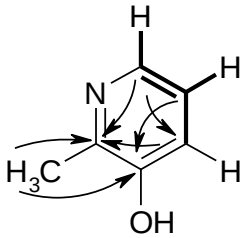
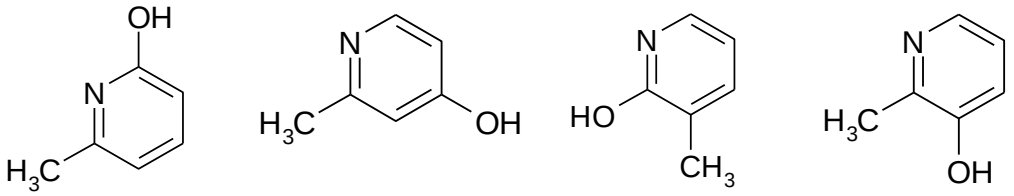


Figure 60: ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of 2-methylpyridin-3-ol (**55**)

By a search in AntiBase based on the spectroscopic data, the four possibilities **52- 55** were taken into consideration. The compound **52** was excluded due to absence of an upfield doublet, and the second structure **53** should show a *m*-coupling, which was not present here; the third structure **54** was also excluded due the absence of the tautomeric pyridone form. The only suitable structure is therefore 2-methylpyridin-3-ol (**55**), which was confirmed by comparing with literature date.[97]



52 **53** **54** **55**

4.3.3 N-(6-Hydroxy-6-methylheptyl)-acetamide

The colourless solid substance **56** was isolated from fraction II as UV inactive solid, which turned to blue with anisaldehyde/sulphuric acid and heating. In the ^{1}H NMR spectrum of **56**, proton signals appeared in the aliphatic region only. The spectrum displayed a methylene group attached to a heteroatom as triplet at δ 3.14, an olefinic sp^2 methyl singlet at δ 1.91, and a singlet of two sp^3 bound methyl groups at δ 1.16. Furthermore, at δ 1.6-1.2 four overlapping methylene signals of an aliphatic chain were observed.

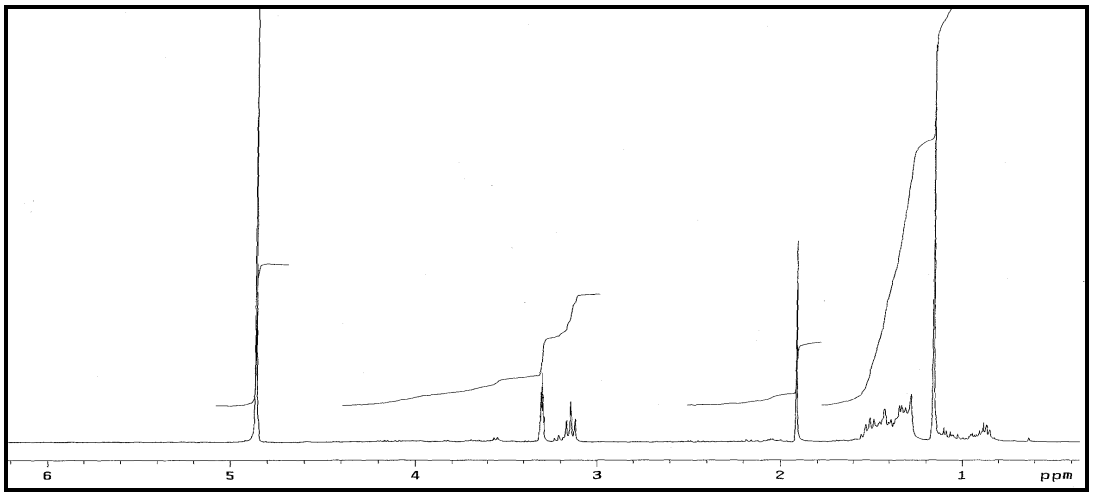


Figure 61: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of N-(6-hydroxy-6-methyl-heptyl)acetamide (**56**)

The ^{13}C NMR spectrum of **56** presented 10 carbon signals, among them a carbonyl carbon at δ 173.2, an oxygenated quaternary carbon at δ 71.4, and five methylene groups at δ 44.7, 40.5, 30.4, 28.6 and 25.1. In the spectrum further two methyl groups appeared at δ 29.2, in addition to an acetyl methyl at δ 22.5.

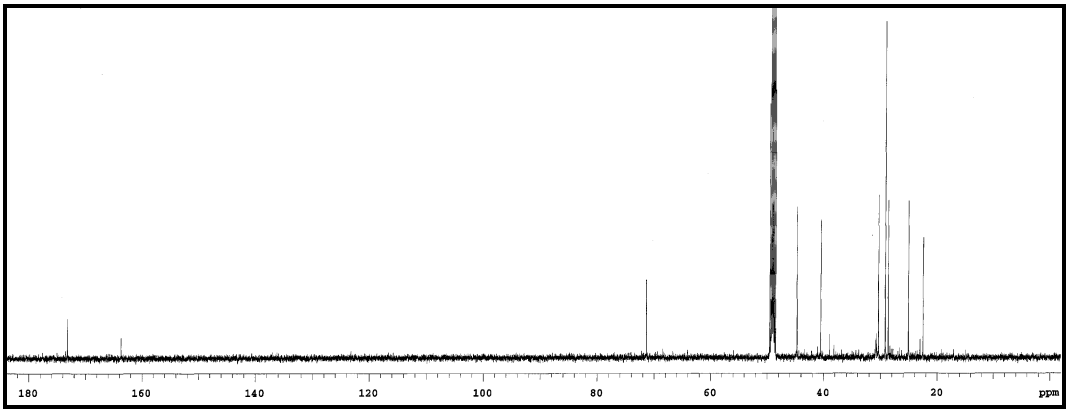
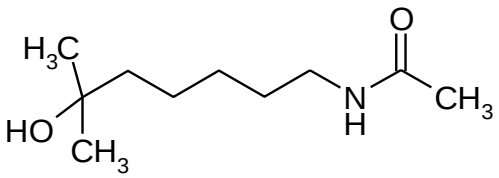


Figure 62: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of N-(6-hydroxy-6-methyl-heptyl)acetamide (**56**)

A search in AntiBase[77] supported by ^{1}H and ^{13}C NMR spectrum data led to N-(6-hydroxy-6-methyl-heptyl)acetamide (**56**). This structure was further confirmed by comparing the spectral data with the authentic data: **56** had been previously isolated in our group from a terrestrial streptomycete by Rahman.[98]



56

4.3.4 Vanillic acid

Fraction II delivered by chromatography on Sephadex LH-20 vanillic acid (**57**) as colourless UV absorbing solid, which did not react with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited two *ortho*-coupled protons at δ 7.34 (J = 1.8, 8.2) and 6.69 (J = 8.1), a *meta*-coupled proton appeared at δ 7.49 (J = 1.6), resulting in a 1,2,4-tri-substituted benzene ring. In the upfield region, a 3H singlet at δ 3.75 offered a methoxy group. The EI mass spectra delivered a molecular weight of 168 Dalton.

Structure **57** was confirmed by comparing the data with authentic spectra. Vanillic acid (**57**) is biosynthetically formed from ferulic acid.[99]

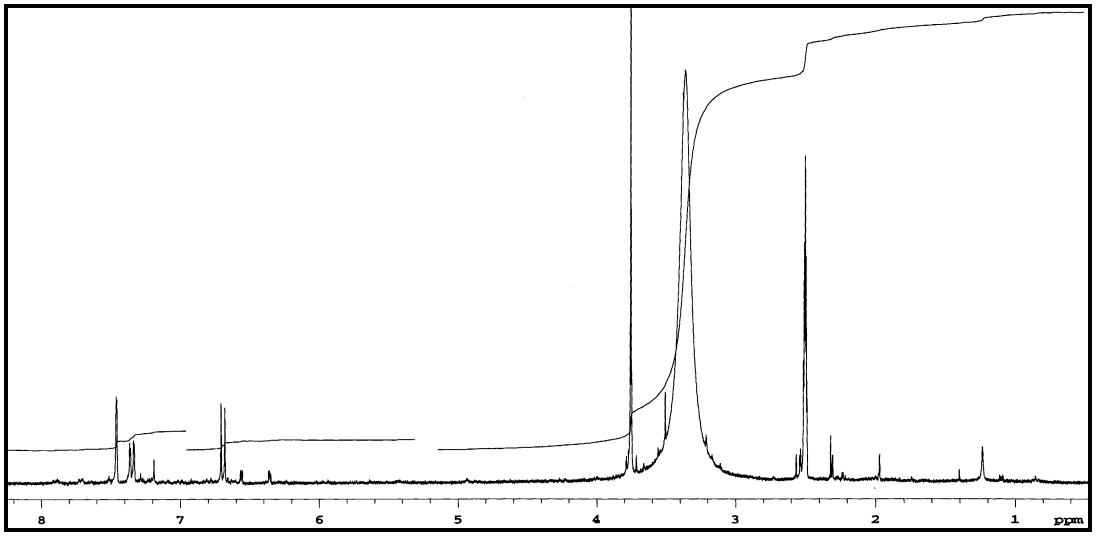
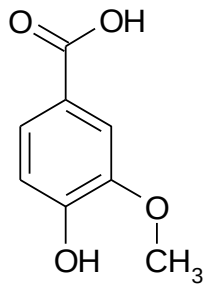


Figure 63: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of vanillic acid (**57**)



57

4.3.5 5'-Acetyluridin

Fraction IV delivered by chromatography on Sephadex LH-20 followed by RP-18 compound **58** as colourless UV absorbing solid, which gave a brownish colour with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of compound **58** displayed two proton signals in the aromatic region as doublets at δ 7.68 (J = 8.1) and 5.72 (J = 8.1) assigned to be due to a α,β-unsaturated carbonyl system. In the aliphatic region, the spectrum displayed a doublet at δ 5.82 (J = 4.0) of an anomeric sugar proton. In addition to methylene protons at δ 4.31, two oxymethines at δ 4.19-4.11, a methine multiplet at δ 4.08, and an sp^2-bound methyl group was observed at δ 2.08; these data resembled the uridine spectrum.

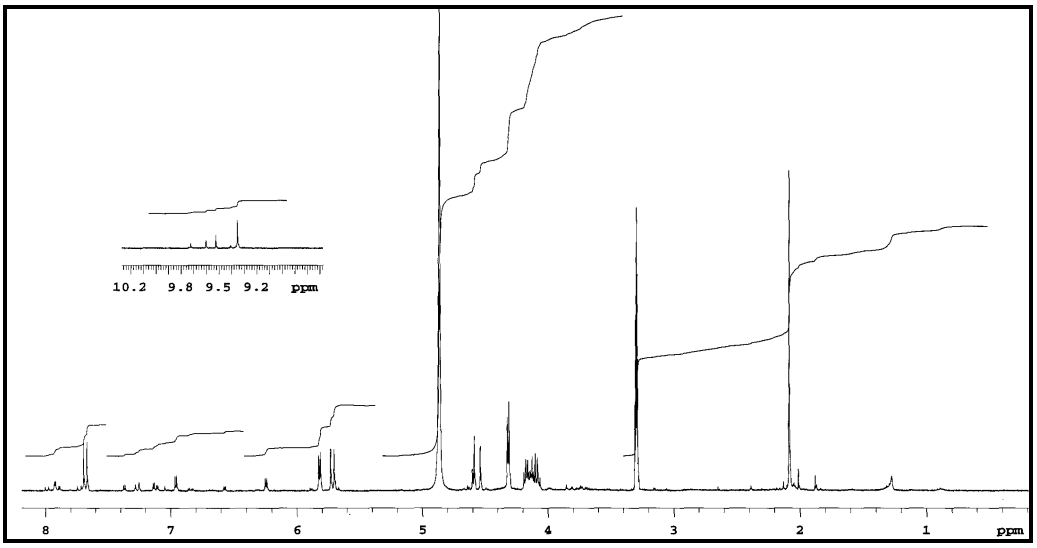


Figure 64: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 5'-acetyluridine (**58**)

In the ^{13}C NMR and HMQC spectra eleven carbon signals were found, among them two downfield signals of quaternary carbons at δ 172.3, and 166.8, suggesting being carbonyl carbons of acid derivatives, and the signal of an oxygenated quaternary carbon at δ 152.8. With the aid of HMQC experiments, two sp^2 carbon signals at δ 142.0 and 102.9 were assigned, which are typical for the uracil moiety. In the aliphatic region, the spectrum exhibited four oxymethine signals at δ 91.8, 75.2, 71.3, and 82.9; additionally, an oxymethylene group was observed at δ 64.7, which could be assigned to a ribosyl residue. Furthermore a methyl signal appeared at δ 20.7.

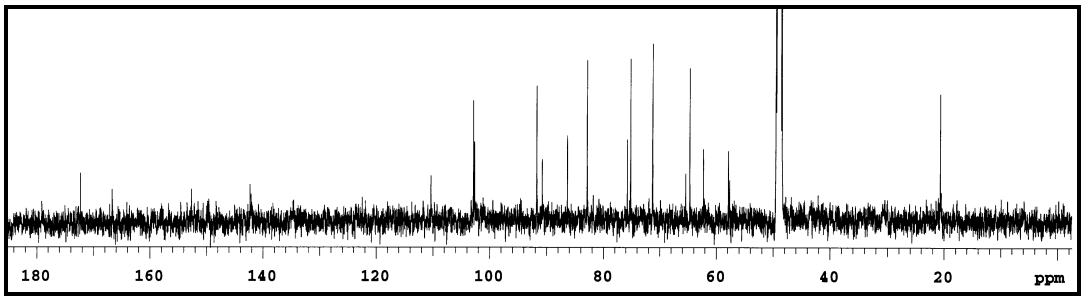
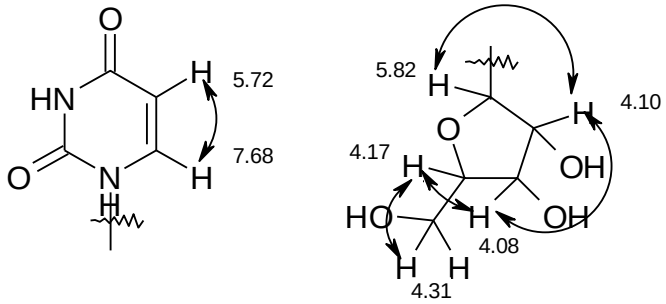


Figure 65: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 5'-acetyluridine (**58**)

The ^{1}H,^{1}H COSY spectrum showed a strong ^{3}J coupling between two protons at δ 7.68 (H-6) and 5.72 (H-5) of the uracil moiety. In addition, a ^{3}J coupling was observed between the anomeric proton 1H at δ 5.82 (H-1') and a signal 1H at δ 4.17 (H-2'). The 1H signal at δ 4.10 (H-4') showed a strong coupling (^{3}J) with the methylene protons at δ 4.31 (H-5'). This led to two fragments **A** and **B**.



A **B**

Figure 66: ^{1}H,^{1}H COSY correlations of 5'-acetyluridine (**58**)

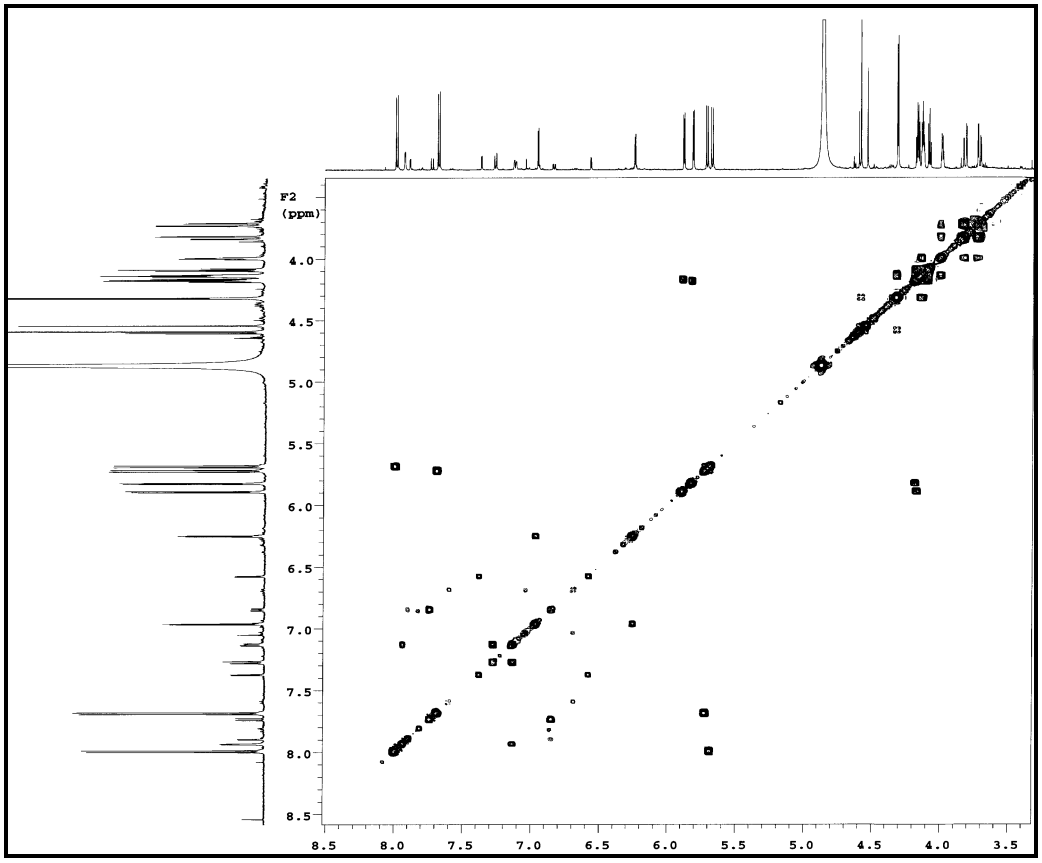


Figure 67: ^{1}H,^{1}H COSY spectrum (CD_3OD, 600 MHz) of 5'-acetyluridine (**58**)

The HMBC spectrum exhibited strong correlations from the methyl protons at δ 2.08 with the ester carbonyl at δ 172.3. The latter also showed a strong ^{3}J coupling with the methylene protons at δ 4.31 (H-5'), confirming the ester group being attached to the methylene group H_2-5'. The methylene group itself showed strong correlation with the oxymethine carbons at δ 82.9 (C-4') and 71.3 (C-3'). In addition, the proton at δ 7.68 (H-6) displayed a ^{3}J coupling with the carbonyls at δ 166.8 (C-4), 152.8 (C-2) and with C-5 (δ 102.9) in the uracil part. It displayed also a three-bond correlation with C-1' (δ 91.8) of the sugar moiety **A** and *vice versa*, confirming the

attachment of the sugar part to uracil at the N-1 position and not at the N-3 position. The latter anomeric proton also displayed a further coupling (^2J) with the methine carbon at δ 75.2 (C-2').

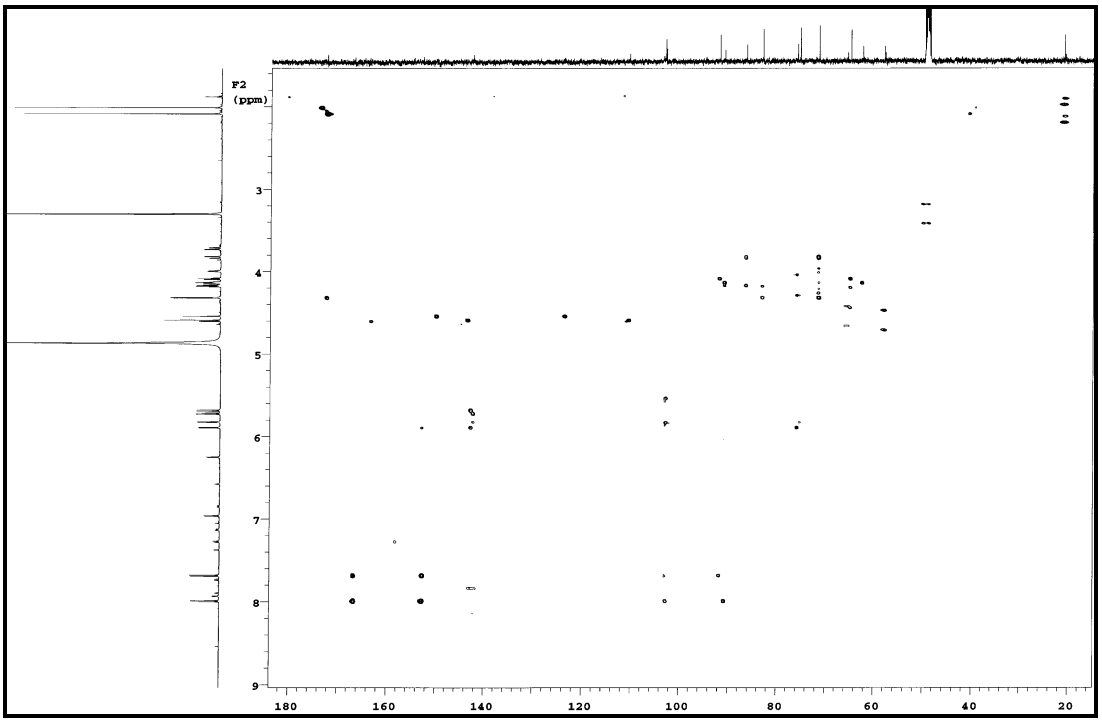
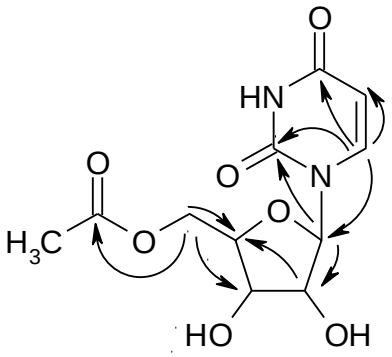


Figure 68: HMBC spectrum (CD_3OD, 600 MHz) of 5'-acetyluridine (**58**)

The ESI mass spectrum of **58** indicated *pseudo*molecular peaks at 309 $[M+Na]^+$, 267 $[(M-COCH_3)+H]^+$, and 511 $[(2M-COCH_3)+H]^+$, which determined the molecular weight as 286 Dalton. A search in AntiBase supported by ^1H, ^{13}C NMR and 2D spectroscopic data led to 5'-acetoxyuridine (**58**). It was isolated recently by Nair in our group from the terrestrial *Streptomyces* sp. GW 7/354.[100]



58

Figure 69: Selected HMBC correlations of 5'-acetyluridine (**58)**

4.3.6 5'-Acetyl-2'-deoxythymidine

The same fraction IV delivered **59** as colourless UV absorbing solid, which gave a brownish colour with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of **59** exhibited in the aromatic region a 1H singlet at δ 7.48, assigned to the β-proton of an α,β-unsaturated carbonyl system. A triplet at δ 6.24 indicated an anomeric proton. In the aliphatic region, two oxymethine protons at 4.34 (H-3'), 4.05 (H-4') were observed. The downfield shift of methylene protons at δ 4.32 and 4.25 (ABX) indicated their connection with an sp^2 carbon or oxygen. Another methylene group was observed at δ 2.24, and finally two methyl singlets appeared at δ 2.08 and 1.88.

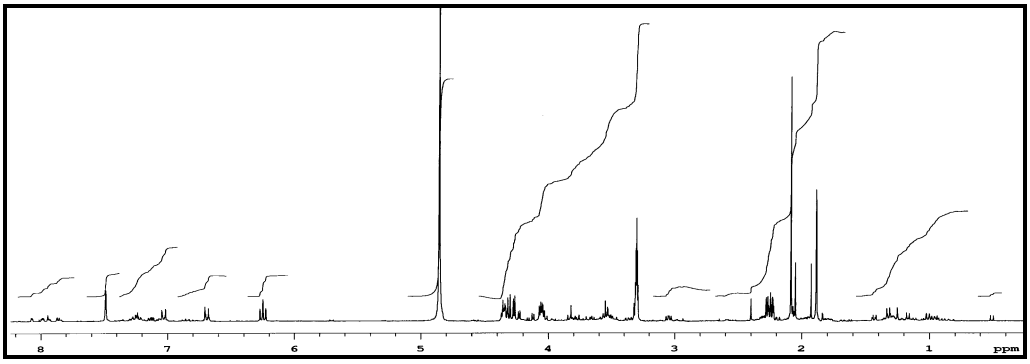


Figure 70: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 5'-acetyl-2'-deoxythymidine (**59**)

The ^{13}C NMR and HSQC spectra revealed twelve carbon signals, among them four quaternary carbons and one aromatic methine at δ 137.5. Additionally five carbon signals were observed in the aliphatic region at δ 86.5, 85.8, 72.3, 65.1 and 40.6, and assigned to a sugar moiety. Finally two methyl carbons at δ 20.7 and δ 12.5 appeared.

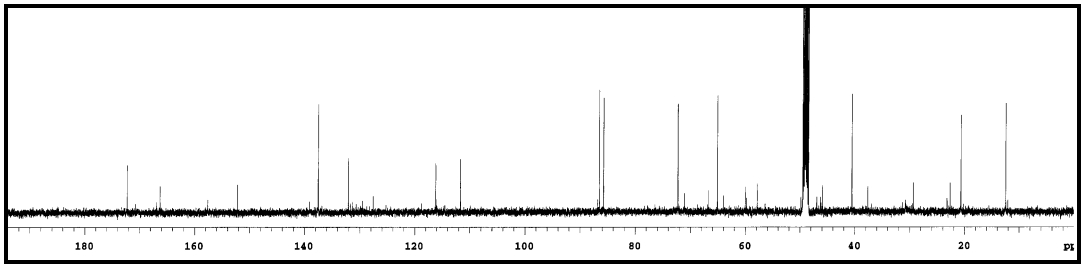
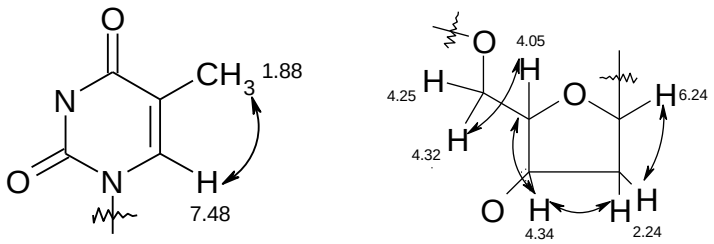


Figure 71: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 5'-acetyl-2'-deoxy-thymidine (**59**)



A **B**

Figure 72: Selected $^{1}H,^{1}H$ COSY correlations of 5'-acetyl-2'-deoxythymidine (**59**)

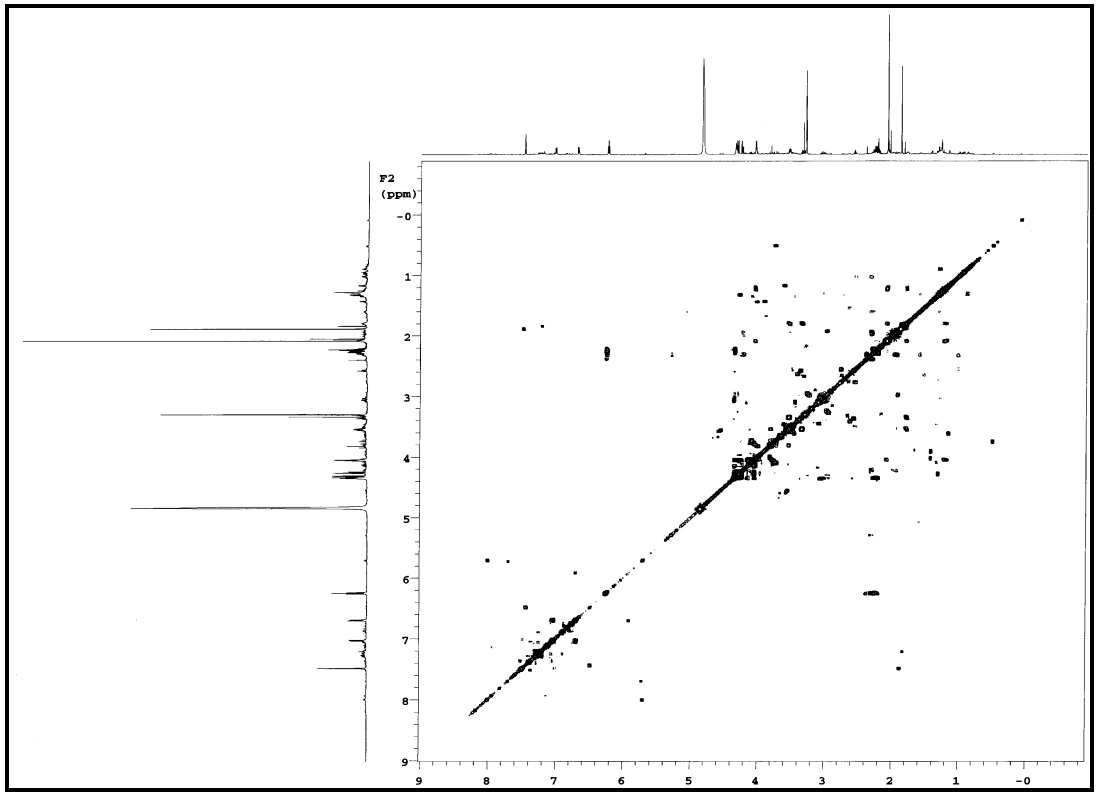


Figure 73: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of 5'-acetyl-2'-deoxythymidine (**59**)

The HMBC spectrum of **59** showed strong correlations from both the methyl group at δ 2.08 and the methylene protons at δ 4.32 and 4.25 with the ester carbonyl at δ 172.3, confirming that the ester group is attached at the carbon number 5' and not at 3'. There was also a three-bond correlation from the methyl signal at δ 1.88 and the singlet at δ 137.5 (C-6) with the carbonyl at δ 166.3 (C-4) and the quaternary carbon at δ 111.7 (C-5). In addition, the anomeric proton at δ 6.24 displayed a strong correlation to the carbonyl at δ 152.2 (C-2) and the aromatic proton at 137.5 (C-6) to confirm again that the sugar moiety is connected at N-1 and not at N-3. Correlations in the sugar moiety were also observed.

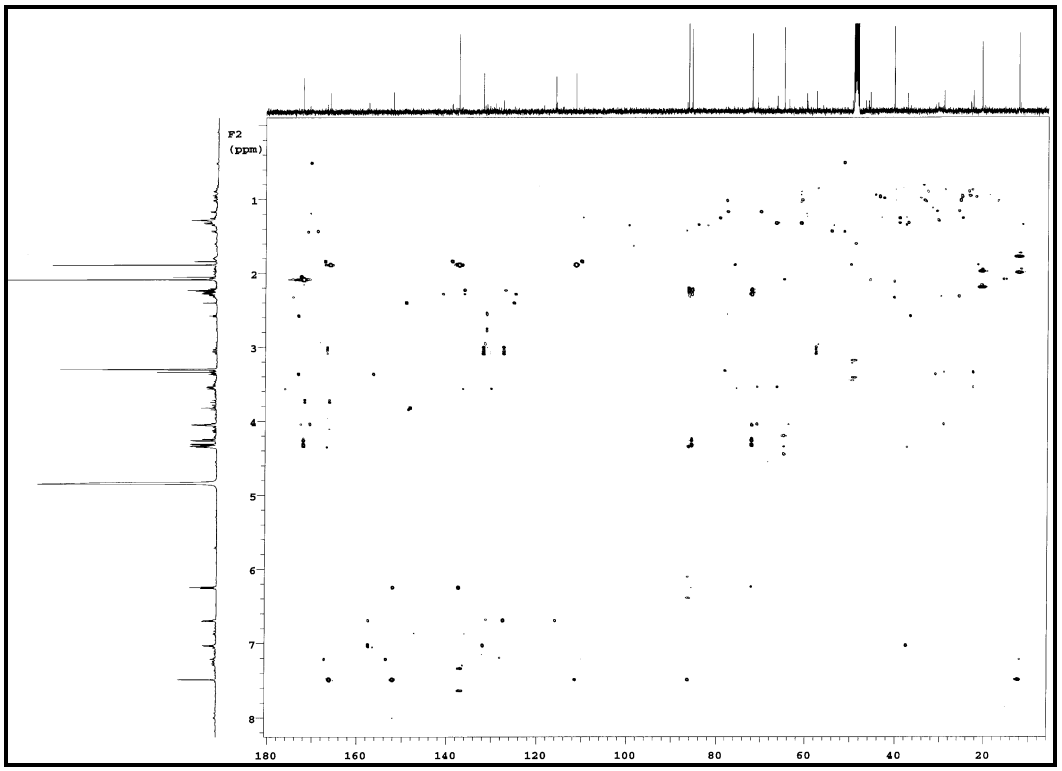
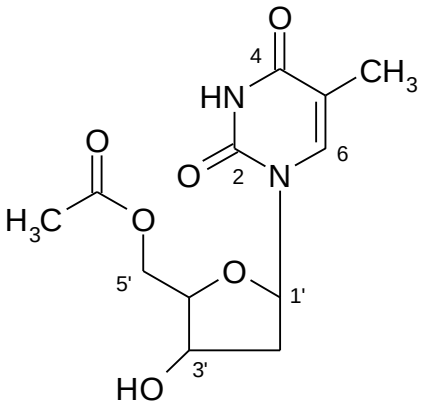


Figure 74: HMBC spectrum (CD_3OD, 600 MHz) of 5'-acetyl-2'-deoxythymidine (**59**)



59

4.4 Terrestrial *Streptomyces* sp. Ank 329

The crude extract of the terrestrial *Streptomyces* sp. Ank 329 showed strong antimicrobial activity against the tested microorganisms, see Figure 251, and TLC analysis showed differently coloured zones with anisaldehyde/sulphuric acid; Ehrlich's reagent indicated the presence of indole derivatives.

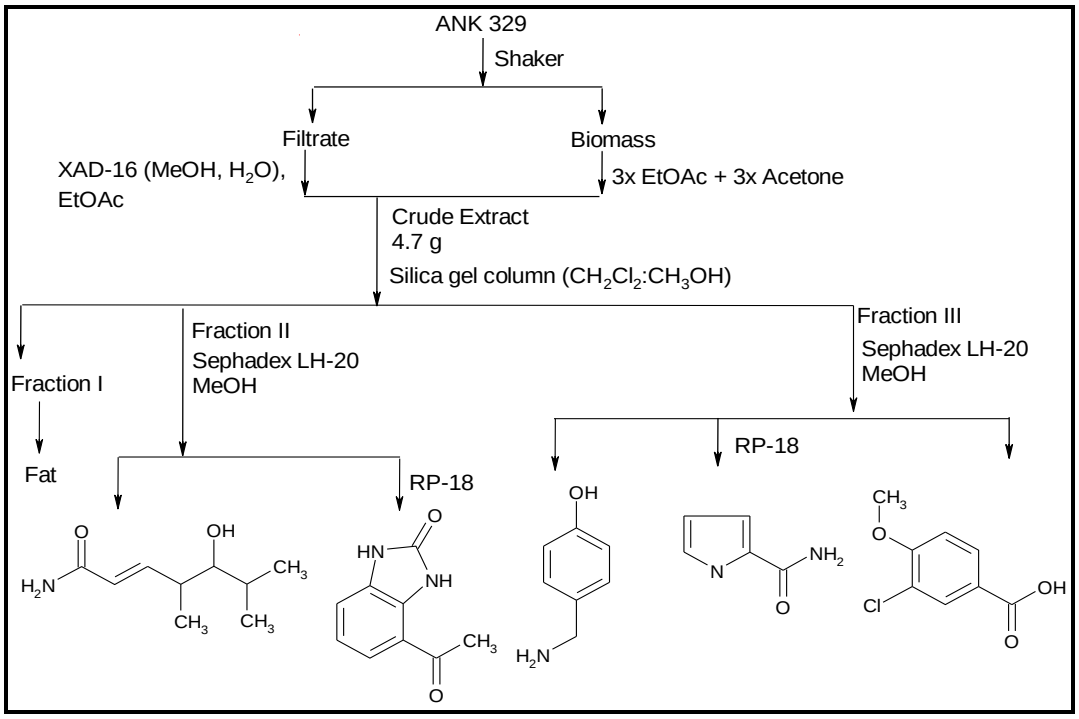


Figure 75: Work-up for scheme for the terrestrial *Streptomyces* sp. Ank 329

4.4.1 (*E*)-4-Methyl-5-hydroxy-6-methyl-2-heptenamide

Compound **60** was isolated as colourless solid from the UV absorbing fraction II, which turned to blue with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **60** exhibited in the olefinic region two protons as multiplet at δ 6.72 (J = 15.5 Hz) and doublet at δ 5.95 (J = 15.6 Hz), suggesting their *trans* orientation in an α,β-unsaturated carbonyl compound. In the aliphatic region, a 1H multiplet at δ 3.16 indicating a methine attached to a heteroatom was observed. Additionally, a multiplet at δ 2.45 and two methyl doublets of an isopropyl group were found at δ 1.68 and 0.90, respectively. ESIMS of **60** showed a *pseudo*molecular ion at *m/z* 194 $[M+Na]^{+}$. The odd mass number was an indication of an odd number of nitrogen atoms in the molecule. HRESIMS established the molecular formula as $C_9H_{17}NO_2$ and confirmed the presence of nitrogen in compound **60**.

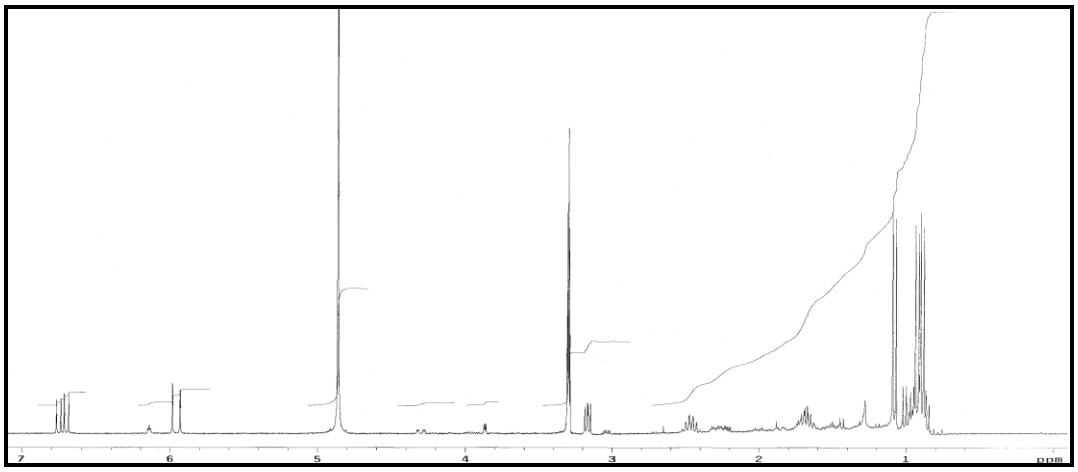


Figure 76: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**)

The ^{13}C NMR spectrum revealed 9 carbon signals, among them one carbonyl of an acid derivative at δ 170.9. In the olefinic region, two methine carbons appeared at δ 149.7 and 123.5, belonging to the double bond of an α,β-unsaturated carbonyl. In the sp^3 region we observed two methine protons, one of them at an oxygenated carbon at δ 80.1 and the other proton signal at δ 41.4. In addition, there were a methyl signal at δ 32.3 and isopropyl signals at δ 20.4, 16.7 and 15.5.

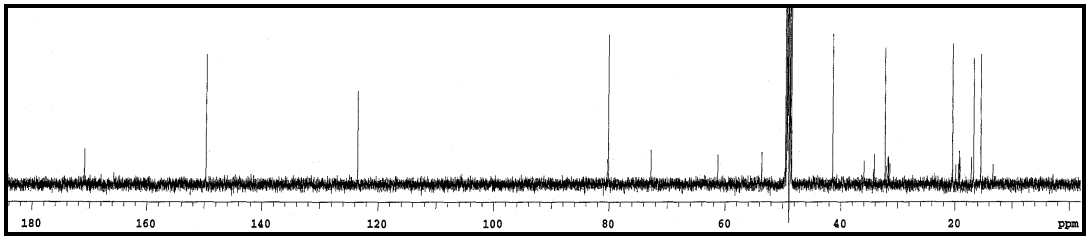


Figure 77: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**)

From ^{1}H,^{1}H COSY correlations, the following fragment was constructed:

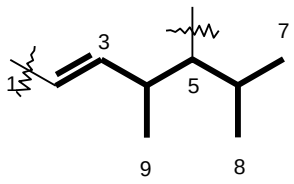
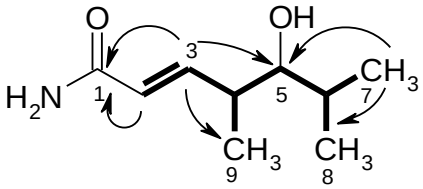


Figure 78: ^{1}H,^{1}H COSY (▬) of (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**)

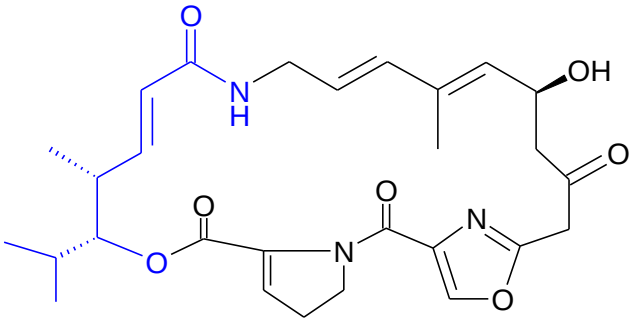
According to the HMBC spectrum, the proton at δ 5.95 showed a ^{2}J coupling with the carbonyl at δ 170.9. The carbonyl also showed ^{3}J correlations with another proton at δ 6.72, which itself showed a ^{3}J coupling with the methyl doublet at δ 1.08 and with the oxygenated carbon at δ 80.1. With the latter carbon, the isopropyl methyls showed correlations as well.



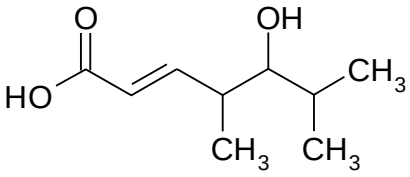
60

Figure 79: Selected ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**)

As there was only one downfield sp^3 signal visible, the nitrogen must be present as an amide, establishing the final structure **60**. The amide is a sub-structure of the madumycins,[101] ostreogrycin A (**61**),[102] the virginiamycins and a few related macrolides. The respective acid **62** [103] is a frequent sub-structure in more than 100 macrolides. In these compounds, the substructures **60** and **61** were always found in the (*E*)-(4*R*,5*R*) configuration, as far as it was determined. The configuration of **60** has not been further investigated.



61



62

By synthesis, (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**) has been obtained according to the following scheme:[104]

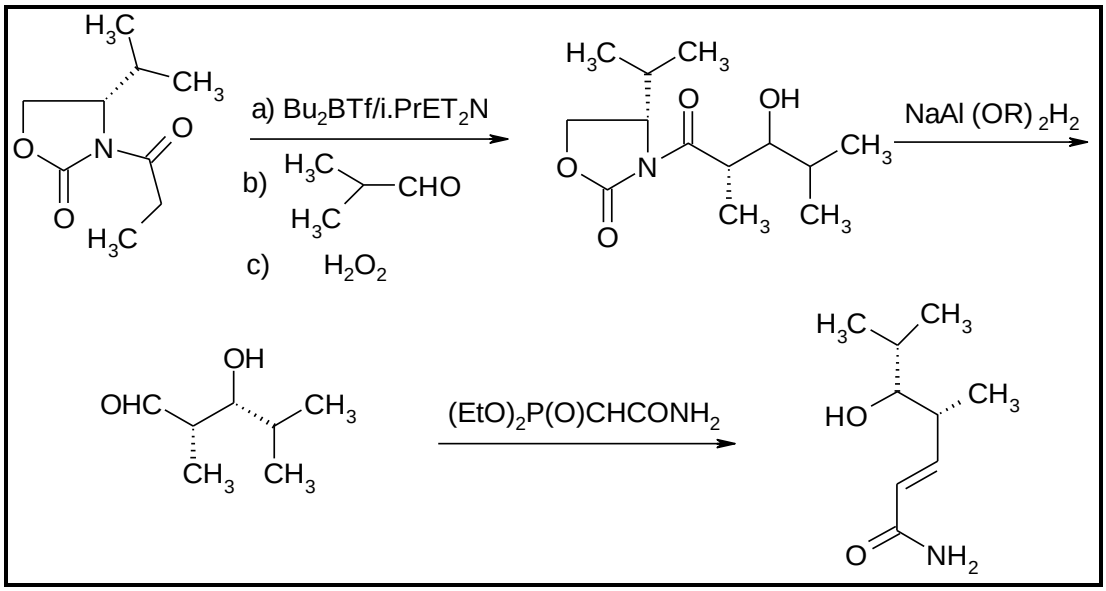
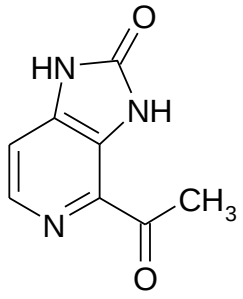


Figure 80: Synthetic scheme for preparation of (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**).

4.4.2 4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one

Compound **63** was isolated as colourless powder from fraction II; the UV absorbing zone turned to yellow when spraying with anisaldehyde/sulphuric acid. ESIMS showed a *pseudo*molecular ion at *m/z* 176 $[M-H]^+$. The odd mass number was an indication of an odd number of nitrogen atoms in the molecule. HRESIMS gave the molecular formula $C_8H_7N_3O_2$ and confirmed the presence of three nitrogen atoms. The ^1H NMR spectrum displayed in the upfield region two doublets at δ 8.21 (J = 5.1 Hz, H-2) and 7.20 (J = 5.1 Hz, H-3) and a methyl singlet attached to an aromatic system or a carbonyl at δ 2.67. A search in AntiBase with these data gave 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**63**). The structure was confirmed by the literature data [98] and also by comparison with authentic spectra.



63

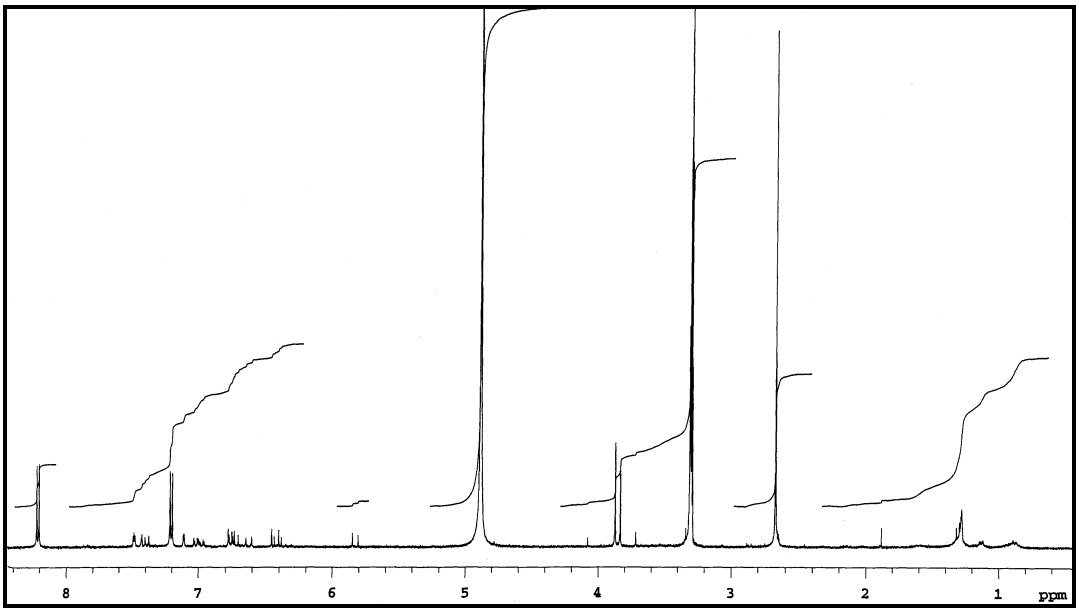


Figure 81: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**63**)

4.4.3 4-Hydroxybenzyl amine

4-Hydroxybenzyl amine (**64**) was isolated as colourless powder in mixture with thymine. The ^{1}H NMR spectrum of **64** showed two *ortho*-coupled proton signals in the aromatic region at δ 7.09 and 6.71, each representing 2H, which suggested the presence of a 1,4-disubstitued benzene ring. In the aliphatic region a 2H singlet was observed at δ 3.38 for a methylene group attached to nitrogen.

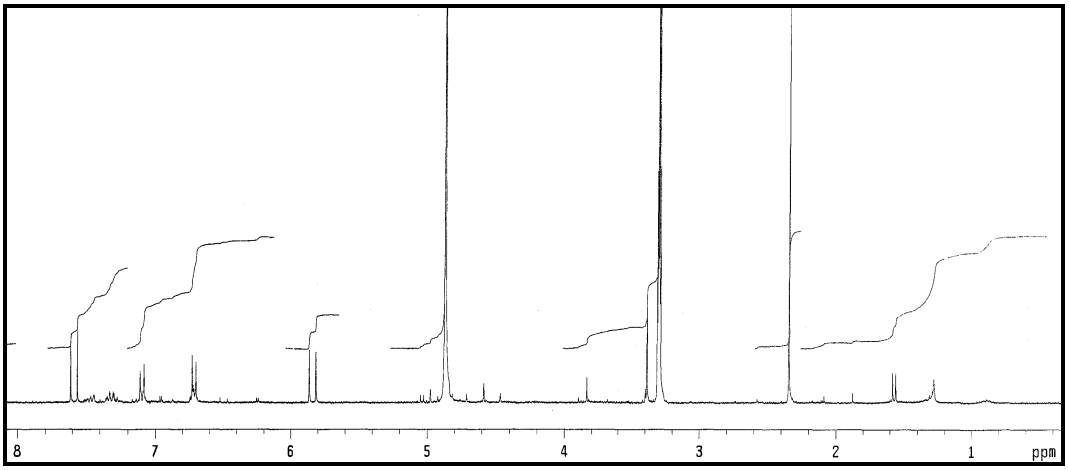
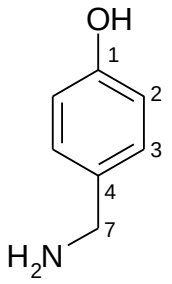


Figure 82: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4-hydroxybenzylamine (**64**)

The ^{13}C NMR spectrum of **64** exhibited five carbon signals, among them one quaternary oxygenated carbon at δ 158.0 (C_q-1), one signal at δ 131.0 representing

two methines (H-3, 5). Another signal at δ 116.3 for further two methines (H-2,6) was visible. One sp^2 quaternary carbon signal was located at 128.0 (C_q-4). Finally a methylene group appeared at δ 42.6. These data pointed to 4-hydroxybenzylamine (**64**)**.** A contamination was identified as N-methyl uracil by shift values and 2D correlations, but could not be separated.

4-Hydroxylbenzylamine has not been isolated from bacteria, but was isolated by Masahiro *et al* [105] from achenes of *Fagopyrum esculentum*, which is widely used in Japan.



64

4.4.4 Indol-3-acetic acid

The ^{1}H NMR spectrum of the compound **65** showed in the aromatic region two 1H doublets at δ 7.53 and δ 7.37, and two 1H triplets at δ 7.09 and 7.01 for a 1,2-disubstituted benzene ring, as well as a singlet at δ 7.17; this signal pattern was indicative for an indole group. In the aliphatic region, a methylene singlet was found at δ 3.64. A search in AntiBase with these data gave indole-3-acetic acid (**65**).

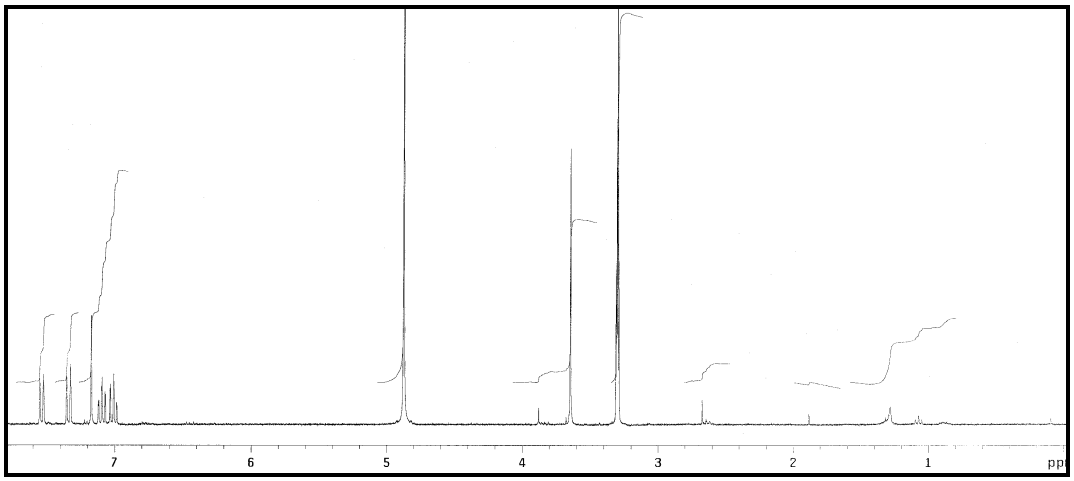
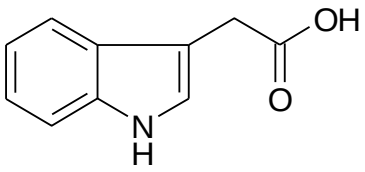


Figure 83: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of indole-3-acetic acid (**65**)



65

4.4.5 3-Chloro-4-methoxybenzoic acid

3-Chloro-4-methoxybenzoic acid (**66**) was isolated as colourless solid from a UV absorbing zone in fraction III, which turned to violet with anisaldehyde reagent. The ^{1}H NMR spectrum of compound **66** gave a 1H double at δ 7.93 (J = 2.0), a doublet of doublet at δ 7.85 (J = 8.5, J = 2.1), and a doublet at δ 7.01 (J = 8.7). This coupling pattern and the coupling constants indicated a 1,2,4-trisubstituted benzene ring. In aliphatic region there was a methoxy group at δ 3.89 visible.

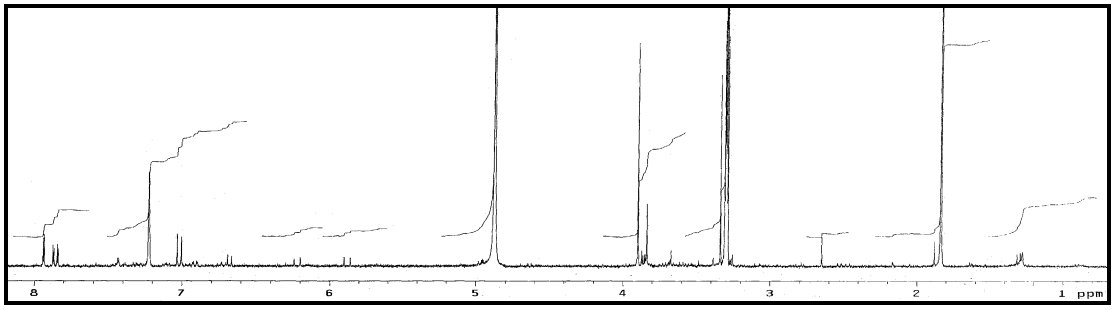


Figure 84: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 3-chloro-4-methoxybenzoic acid (**66**)

The ^{13}C NMR spectrum exhibited seven carbon signals, among them the carbonyl signal of an acid derivative at δ 173.6, an oxygenated and a non-oxygenated quaternary carbon at 157.9 and 122.4, respectively, three methine carbons at δ 132.2 (CH-2), 130.3, 112.0, and a methoxy carbon at δ 56.6.

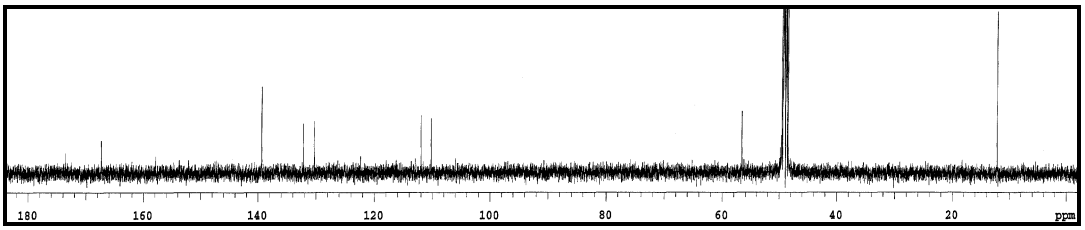
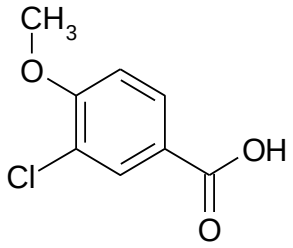


Figure 85: ^{13}C NMR spectrum (125 MHz, CD_3OD) of 3-chloro-4-methoxybenzoic acid (**66**)

The ESI mass spectrum indicated the *pseudo*molecular peak at *m/z* 185 $[M-H]^+$, and HRESIMS established the molecular formula as $C_8H_7O_3Cl$. A search in Anti-Base[77] supported by ^1H, ^{13}C NMR, and MS spectroscopic data led to 3-chloro-4-methoxybenzoic acid (**66**). It was further confirmed by the literature data.



66

Initially this compound **66** was isolated from basidiomycetes, which are capable of producing high concentrations of chloroaromatic metabolites.[106] One of them is *Lepista nuda*, forming fairy rings in coniferous forests.[107] A contamination was identified as thymine by shift values and 2D correlations, but could not be separated.

4.4.6 Pyrrole-2-carboxamide

Pyrrole-2-carboxamide (**67**) was isolated as colourless powder, which formed a UV absorbing band at 254 nm on TLC and turned to yellow with anisaldehyde/sulphuric acid. The ^1H NMR spectrum of compound **67** showed three aromatic protons at δ 6.82 (J = 5.4 Hz), 6.73 (J = 4.6 Hz), and 6.05 (J = 4.9 Hz). From the small coupling constants (5.4 - 4.6 Hz) it was plausible that the compound contained a small ring like a five membered heterocycle. Furthermore one broad proton at δ 11.30 according to an NH group was observed.

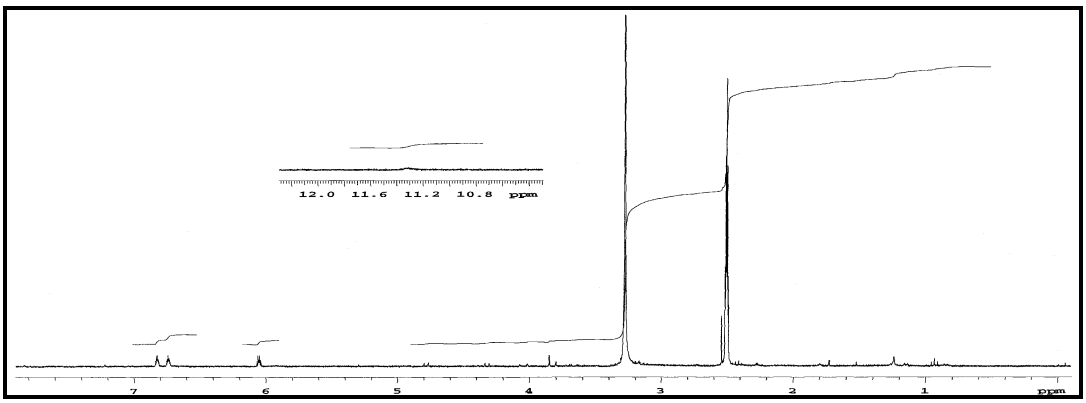


Figure 86: ^1H NMR spectrum (DMSO-d_6, 300 MHz) of pyrrole-2-carboxamide (**67**)

The ^{13}C NMR spectrum of the compound **67** showed five carbon signals. The first signal at δ 162.0 might be due to a carboxamide; three methine carbons were recorded at δ 121.1, 110.4 and 108.3 and the fifth carbon was likely to be a quaternary carbon at δ 126.1.

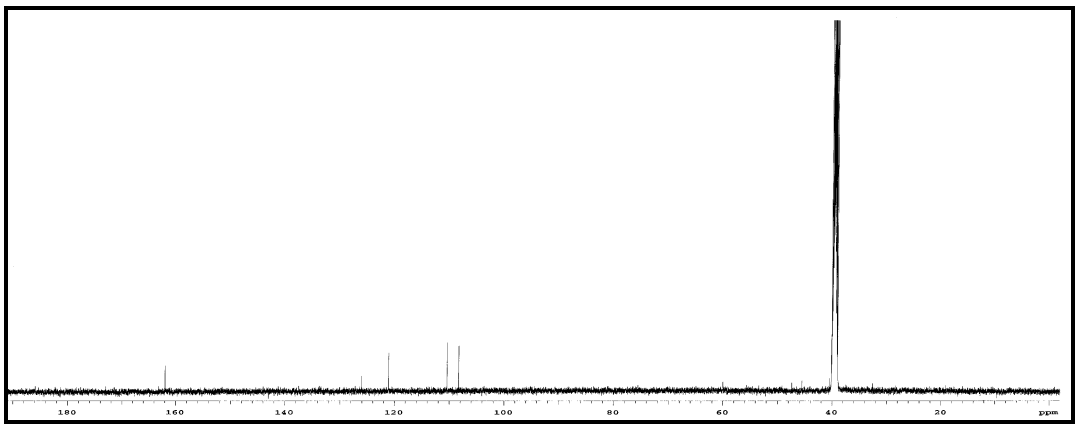
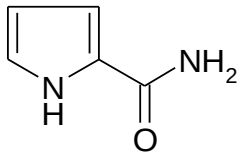


Figure 87: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of pyrrole-2-carboxamide (**67**)

The molecular weight of pyrrole-2-carboxamide (**67**) was determined as 110 Dalton by EIMS, and the base peak as well as a fragment at *m/z* 66 confirmed a pyrrole ring. A search in AntiBase[77] and Scifinder using the ^{1}H NMR, ^{13}C NMR data as well as the molecular weight revealed pyrrole-2-carboxamide. It is a known synthetic compound[108] and has been confirmed by comparison of our spectroscopic data with the literature values.[109] Pyrrole-2-carboxamide has been isolated from the tropical marine sponge *Agelas oroides.*



67

4.5 Terrestrial *Streptomyces* sp. ANK 312

The crude extract of the terrestrial *Streptomyces* sp. ANK 312 showed biological activity only against *Staphylococcus aureus*; the TLC analysis exhibited zones showing blue colour reactions with anisaldehyde/sulphuric acid.

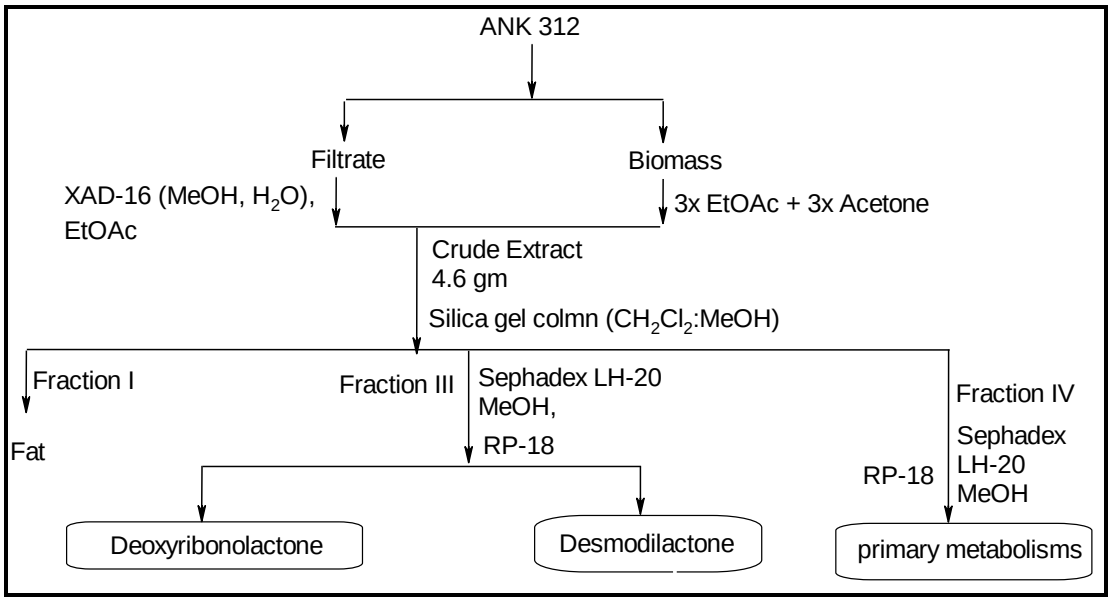


Figure 88: work-up scheme terrestrial S*treptomyces* sp. ANK 312

4.5.1 Deoxyribonolactone

Deoxyribonolactone (**68**) was isolated as brown oily substance, which turned to pink with anisaldehyde spraying reagent. The molecular weight was deduced by ESI mass as 132 Dalton. HRESIMS established the empirical molecular formula as $C_5H_8NaO_4$. The ^{1}H NMR spectrum showed no proton signals in the aromatic region, but two oxymethine protons in the aliphatic region at δ 4.42, 4.36. The downfield shift of two additional ABX methylene signals at δ 3.76/3.68 (H_2-6) and δ 2.91/2.37 (H_2-3) indicated that the respective carbons were in connection with sp^2 centres or heteroatoms.

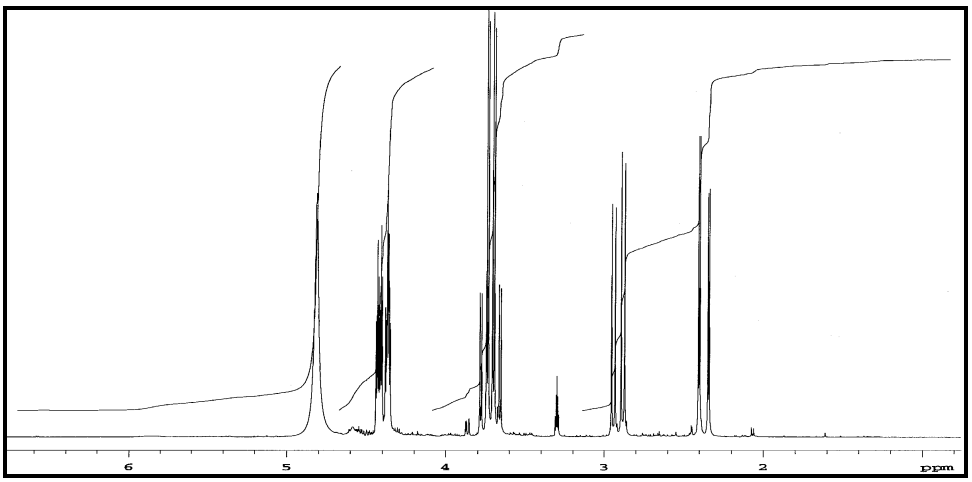


Figure 89: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of deoxyribonolactone (**68**)

The ^{13}C NMR/ATP spectrum of **68** showed only five carbon signals including a carbonyl at δ 178.6 of an amid, acid or ester. Additionally, two oxymethine signals at δ 90.1 and 69.6 as well as two methylene carbons at δ 62.5 and 39.1 were assigned according to the HMQC spectrum.

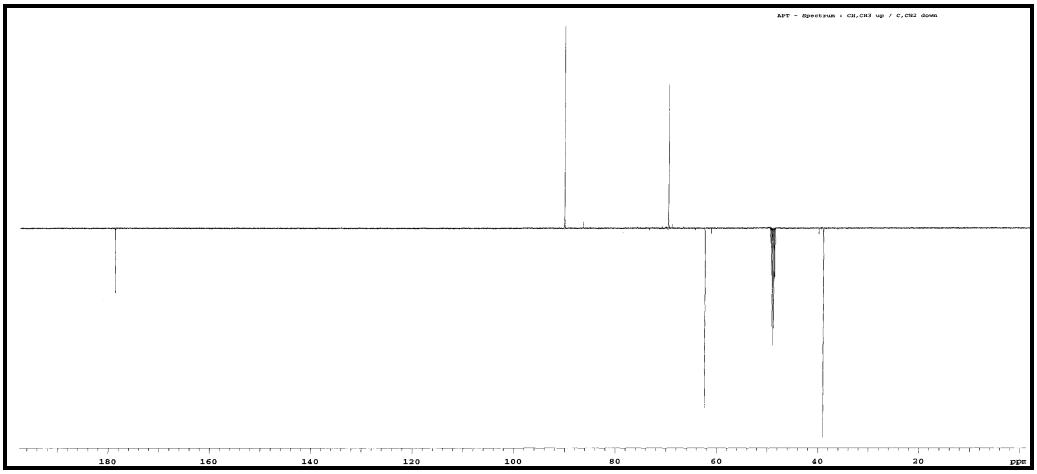


Figure 90: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of deoxyribonolactone (**68**)

The ^{1}H,^{1}H COSY spectrum showed a strong coupling between the oxymethine proton at δ 4.42 and the oxymethylene at δ 3.76/3.68 (H_2-3) and ^{3}J couplings with the oxymethine at δ 4.36. The latter showed also ^{3}J correlations with the ABX methylene protons at δ 2.91 and 2.37.

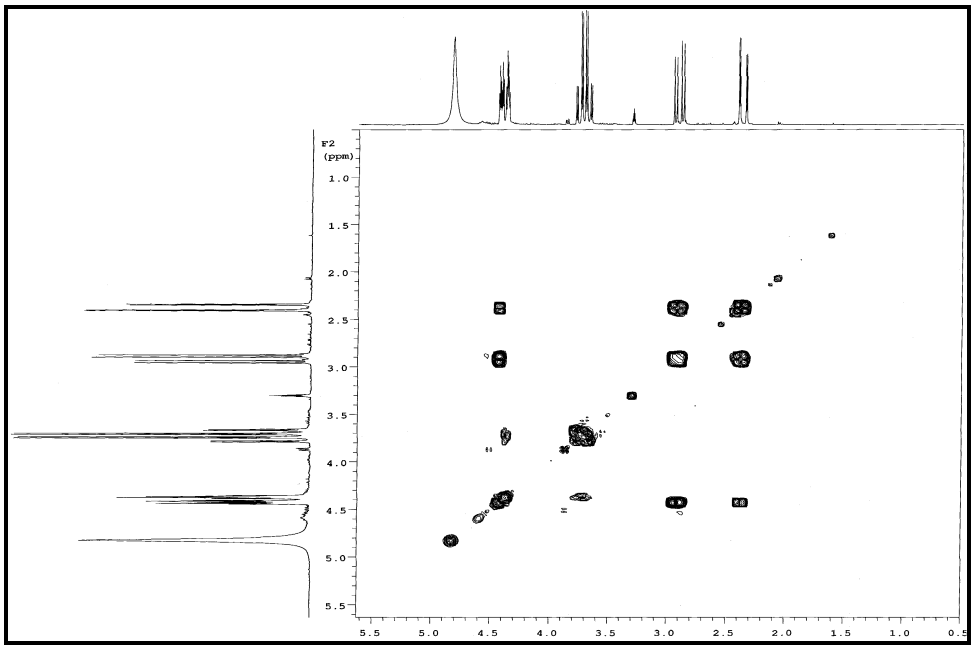


Figure 91: ^{1}H,^{1}H COSY spectrum (CD_3OD, 300 MHz) of the deoxyribonolactone (**68**)

In the HMBC spectrum, two methines at δ 4.42 (H-4) and 4.36 (H-5) and the methylene group at δ 2.91 and 2.37 showed strong correlations with the carbonyl at δ 178.6 to establish a butanolide. The methylene protons at δ 2.91 and 2.37 showed strong couplings to both oxymethine protons at δ 4.42 (H-4) and 4.36 (H-5). Further correlations confirmed the structure as deoxyribonolactone (**68**) or a stereoisomer thereof.

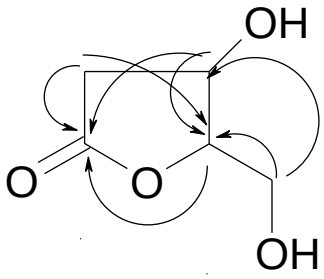


Figure 92: Selected HMBC (→) couplings of deoxyribonolactone (**68**)

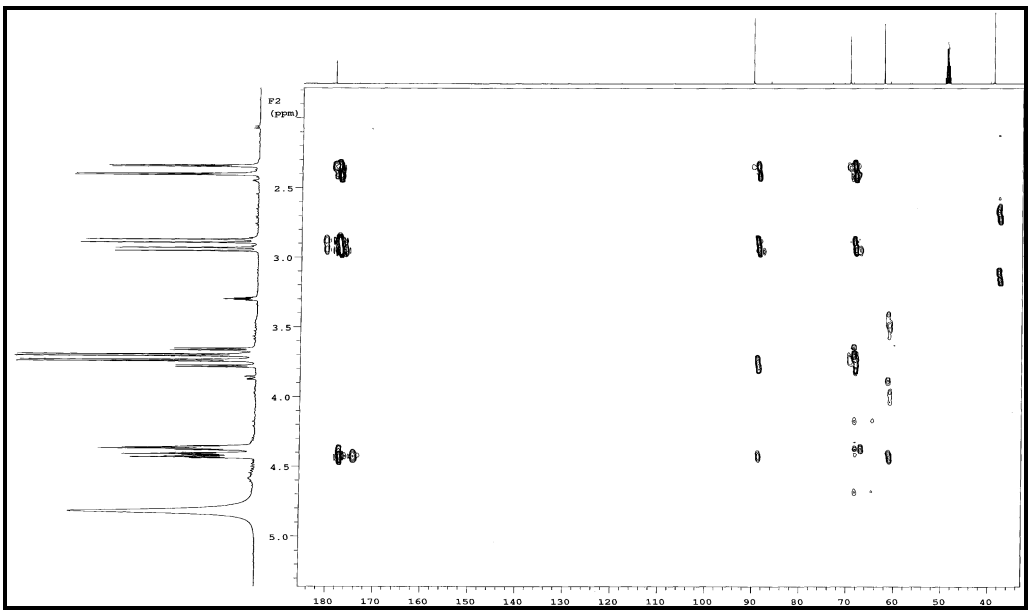
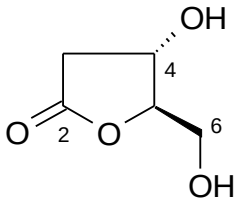


Figure 93: HMBC spectrum (CD_3OD, 75 MHz) of deoxyribonolactone (**68**)

The small coupling constant for H-4 (J = 2.3) and H-5 (J = 2.3) indicated the *trans*-configuration, i.e. deoxyribonolactone. Compound **68** was isolated from the acetone extract of the leaves of *Aristolochia arcuata*[110] and by Ming *et al.* from the roots of *Clematis chinensis* [111]. Now it was isolated from bacteria for the first time.



68

4.5.2 Desmodilactone

Compound **69** was isolated as colourless UV inactive substance, which gave a blue colour by spraying with anisaldehyde/sulphuric acid and heating. The molecular weight was deduced as 171 Dalton by positive ESIMS, showing a *pseudo*molecular ion peak of *m*/*z* 194 $[M+Na]^+$. HRESIMS delivered the molecular formula of $C_8H_{13}NO_3$.

The ^1H NMR spectrum displayed two protons attached to hetero-atoms; the first proton appeared as doublet centred at δ 4.65 (H-3), while the second one gave a multiplet at δ 4.36 (H-5); another methine multiplet was observed at δ 2.38 (H-5). A methyl singlet at δ 2.02 was supposed to be attached to an sp^2 carbon and two methyl groups gave doublets at δ 1.40 and 0.96.

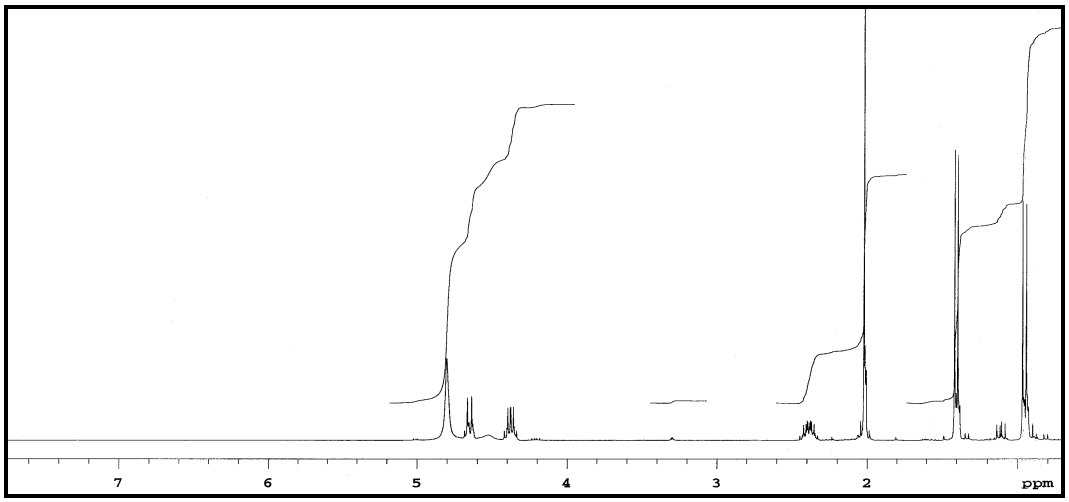


Figure 94: ^1H NMR spectrum (CD_3OD, 300 MHz) of desmodilactone (**69**)

The ^{13}C NMR and HMQC spectra indicated the presence of 8 carbon signals as expected, of which two at δ 176.6 and 173.3 were due to carbonyls of acid derivatives. Two oxymethine carbons at δ 84.0 and 53.4 were observed, along with three methyl signals at δ 29.0, 19.9, and 12.7.

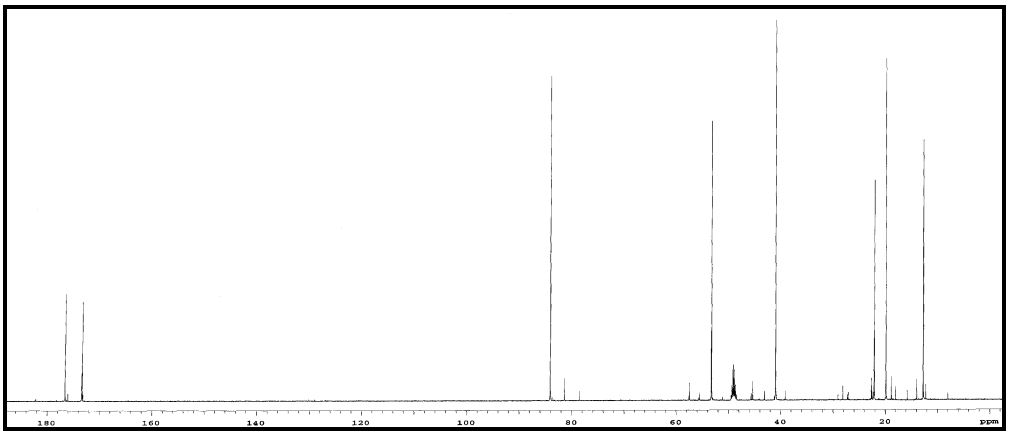


Figure 95: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of desmodilactone (**69**)

A search in AntiBase[77] supported by ^{1}H, ^{13}C NMR data did not afford hits, indicating a new natural product from bacteria. The $^{1}H,^{1}H$ COSY spectrum exhibited strong correlations between the methyl doublet at δ 1.40 and a methine signal at δ 4.65, which correlated with another proton at δ 2.38. The latter one exhibited two further correlations to the methyl doublet at δ 0.96 and to the methine doublet at δ 4.36. The COSY correlations gave the following connections Figure 96

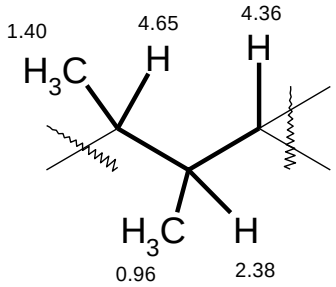


Figure 96: Selected $^{1}H,^{1}H$ COSY (▬) of desmodilactone (**69**)

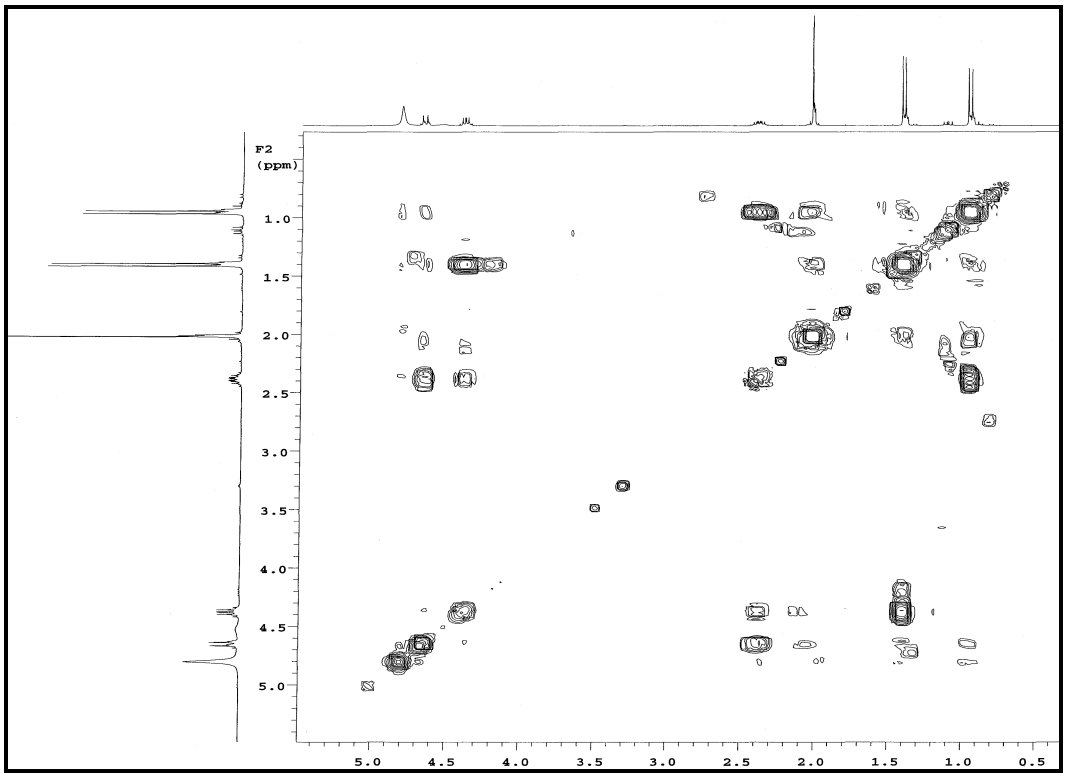
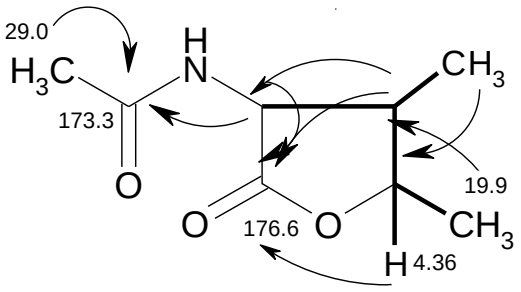


Figure 97: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of desmodilactone (**69**)

The HMBC spectrum exhibited strong correlations from both the methyl singlet at δ 29.0 and the methine proton at δ 4.65 to a carbonyl signal at δ 173.3. The latter proton displayed another correlation with a second carbonyl at δ 176.6 that itself showed a ^{3}J correlation with a methine proton at δ 4.36. All correlations confirmed the structure as lactone **69**.



69

Figure 98: selected ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of desmodilactone (**69**)

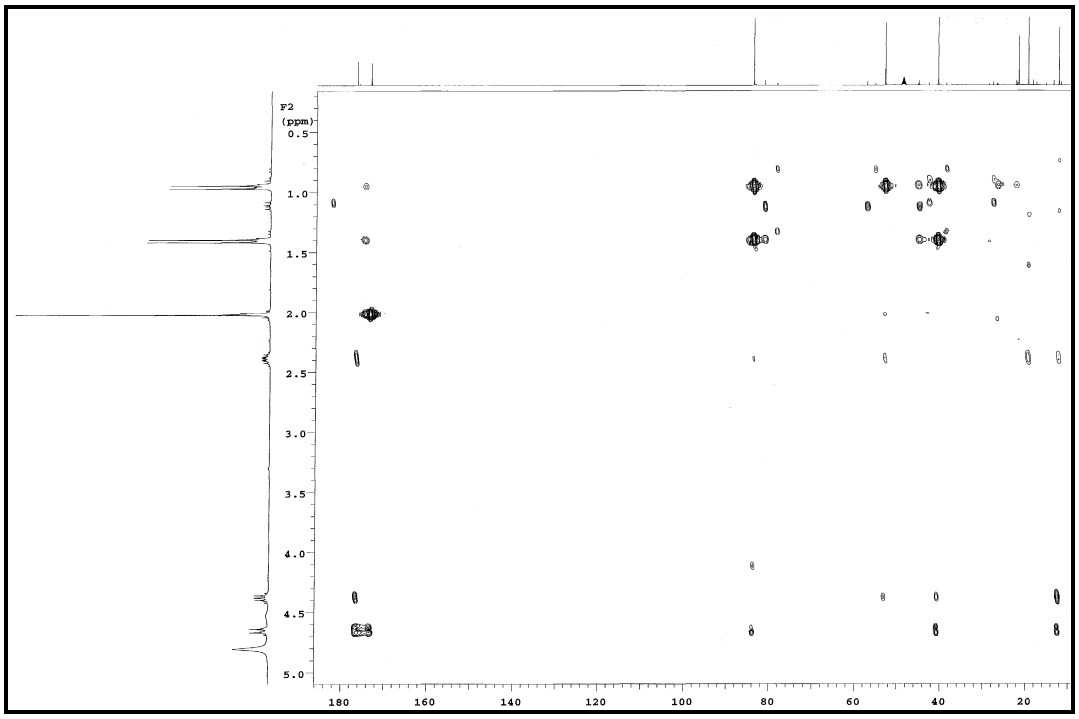
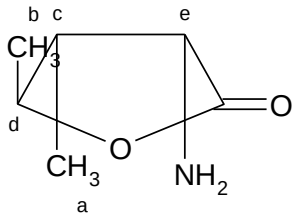
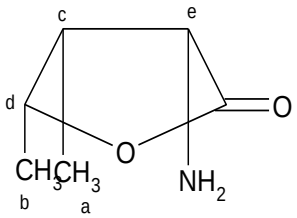


Figure 99: HMBC spectrum (CD_3OD, 75 MHz) of desmodilactone (**69**)

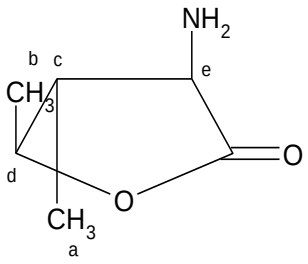
Compound **69** was thereby identified as desmodilactone, which had been isolated from the aerial parts of *Desmodium styracifolium*, but is new from bacteria.[112] There are four related diastereomeric lactones available from literature identified as (2*S*, 3*R*, 4*S*)-**70**, (2*S*, 3*R*, 4*R*)-**71** , (2*R*, 3*S*, 4*S*)-**72** and (2*S*, 3*S*, 4*S*)-**73**.[113]



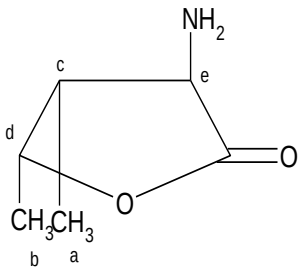
70



71



72



73

Table 11: ^{1}H NMR values for the related isomers

Lacton	a	B	c	d	e
70	1.10	1.37	2.55	4.51	4.49
71	0.91	1.28	2.85	4.83	4.64
72	1.23	1.36	2.31	4.28	4.11
73	1.20	1.25	2.94	4.82	4.24

4.6 Terrestrial *Streptomyces* sp. ANK 320

The crude extract of the terrestrial *Streptomyces* sp. ANK 320 showed weak biological activity against *Mucor miehei* and *Artemia salina* and the TLC analysis exhibited two UV active zones, which turned to yellow and red with anisaldehyde/sulphuric acid.

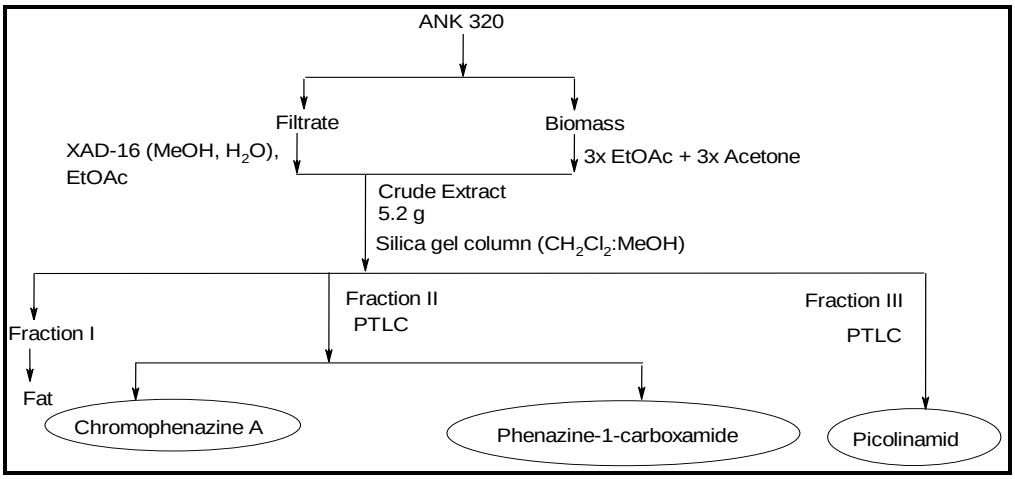


Figure 100: Work-up scheme for terrestrial *Streptomyces* sp. ANK 320

4.6.1 Chromophenazine A

Chromophenazine A (**74**) was isolated as an orange solid, which showed a UV absorbing band at 254 nm, which turned blue on the TLC plate on spraying with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum exhibited in the aromatic region three protons at δ 8.82 (d, J = 8.2), 8.21 (d, J = 2.0) and 8.03 (dd, ^{4}J = 1.6, ^{3}J = 7.9 Hz). The coupling pattern and the coupling constants indicated a 1,2,4-trisubstituted benzene ring. In addition at δ 7.58 and 7.40 four protons overlapped, indicating a 1,2-disubstitued benzene ring. A 1H double bond singlet appeared at δ 6.17. Moreover, a 1H multiplet at δ 5.18 suggesting an olefinic or oxymethine proton was observed. In addition, the AB part of an ABX system at δ 4.83 (J = 6.1 Hz) of a meth-

ylene group was seen. The downfield shift of the methylene protons indicated the neighbourhood flanking by sp^2 carbons or the attachment to heteroatoms. The spectra exhibited another two methyl singlets (δ 1.94 and δ 1.79) with a small allylic coupling (J = 1 Hz), indicative of their neighbourhood to an olefinic proton as in an isopropenyl group. Hence, a prenyl residue [-CH_2-CH= C (CH_3)2] was recognized.

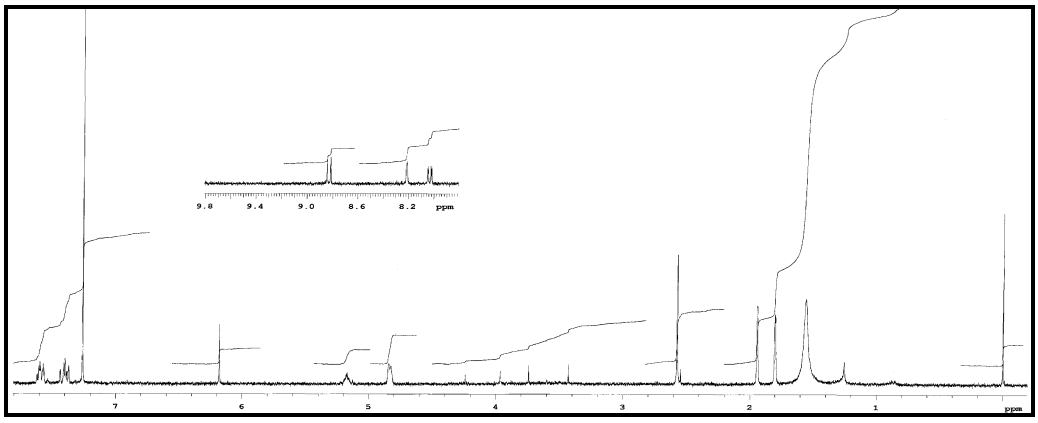
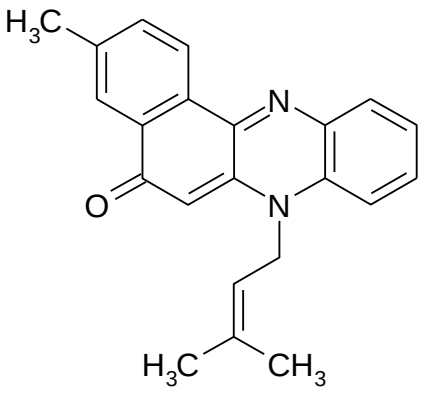


Figure 101: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of chromophenazine A (**74**)

Table 12: ^{1}H and ^{13}C NMR data ($CDCl_3$, 300, 125MHz) of chromophenazine A (**74**)

	chromophenazine A (**74**)	
No	δ_H (mult. J in [Hz])	δ_C
1	7.96 (dd, 7.9, 1.5)	130.8
2	7.36 (t , 7.9)	123.6
3	7.56 (t, 7.9)	130.9
4	7.32 (d, 7.9)	113.4
4a	-	131.3
5a	-	139.2
6	6.12 (s)	99.4
7	-	181.9
7a	-	132.7
8	8.14 (d, 1.7)	125.4
9	-	141.7
9-Me	2.52 s	21.9
10	7.54 (dd, 8.2, 1.7)	131.8
11	8.74 (d, 8.2)	125.0
11a	-	129.5
11b	-	146.9
12a	-	135.0
1'	4.76 (d, 5.4)	45.7
2'	5.14 m	116.5
3'	-	138.2
4'	1.77 s	25.6
5'	1.91 s	18.7



74

The molecular weight of **74** was established by ESI mass spectrum, which showed a *pseudo*molecular ion at *m/z* 329 $[M+H]^+$, corresponding to a molecular weight of 328 Dalton; the molecular formula was established as $C_{22}H_{20}N_2O$ by HRESIMS, containing fourteen double bond equivalents. According to these spectroscopic and mass data, the compound was identical with chromophenazine A (**74),** which had been isolated in parallel in our group from strain ANK 315.[114]

4.6.2 Phenazine-1-carboxamide

Phenazine-1-carboxamide (**75**) was isolated from fraction FII as pale yellow solid. In the ^1H NMR spectrum of **75,** seven aromatic proton signals were observed; three at δ 8.83 (dd, H-2), 7.96 (dd, H-3), 8.42 (dd, H-4) were adjacent in a first spin system, and four protons at δ 8.26 (m, H-6), 8.26 (m, H-9), 7.93 (dd, H-7) and 7.88 (dd, H-8) belonged to a 1,2-disubstituted benzene ring. In the aliphatic region no signal was observed. The ESI mass spectrum delivered a *pseudo*molecular ion peak at *m/z* 246 for $[M+Na]^+$, which gave a molecular weight of 223 Dalton. The HRESI mass spectrum established the molecular formula $C_{13}H_9N_3O$. A search in AntiBase by comparing the spectroscopic information led to phenazine-1-carboxamide (**75),** a compound known from the bacterium *Pseudomonas chlororaphis* PCL1391 and found to be useful to biocontrol root rot of tomato.[115] It was also isolated from an Antarctic sponge-associated bacterium *Pseudomonas aeruginoss.* Phenazine carboxamide (**75**) and phenazine-1-carboxylic acid were found to be active against *Bacillus cereus (*MIC by disk assay < 0.5 μg/ml); phenazine-1-carboxylic acid is more potent than phenazine carboxamide. Both compounds were less active against *M. luteus* and *S. aureus (*MIC > 5 μg/ml).[116]

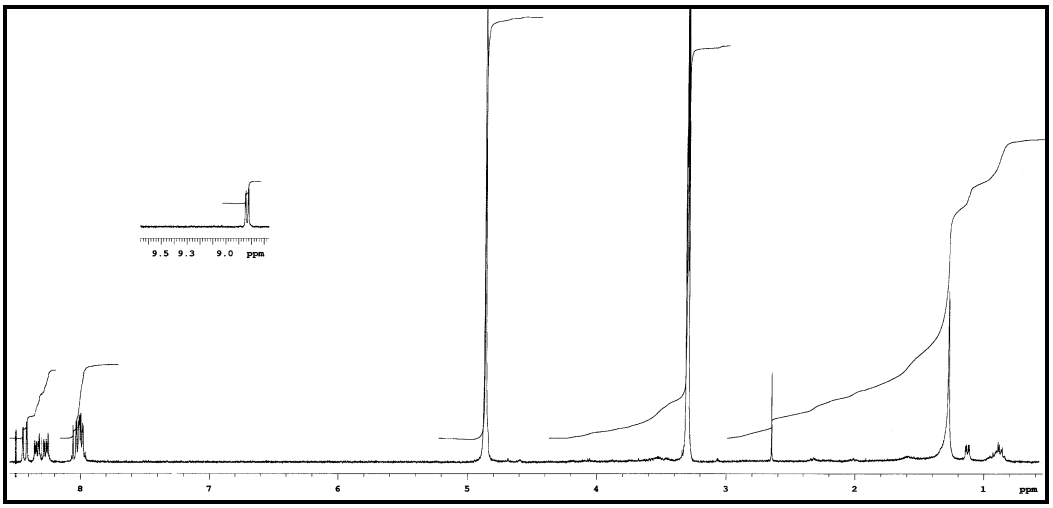
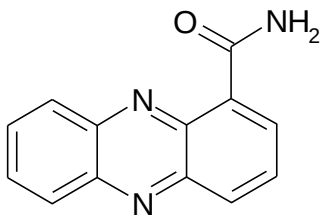


Figure 102: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of phenazine-1-carboxamide (**75**)



75

4.6.3 Picolinamid

Picolinamid (**76**) was isolated as yellow crystals from the middle polar fractions as UV absorbing substance, which turned to yellow with anisaldehyde reagent. The molecular weight was established by the EI mass spectrum as 122 Dalton. The ^{1}H NMR spectrum of compound **76** displayed in the aromatic region one proton as doublets of doublet at δ 8.62 (J = 6.4, J = 4.8 Hz, H-6), indicative of an attachment in a hetero cycle. Two further protons appeared as doublets of triplets at δ 8.09 (J = 7.8, J = 2.1 Hz, H-3) and 7.94 (J = 7.7, J = 1.7 Hz, H-4), in addition to doublets of a doublet at δ 7.53 (J = 7.6, J = 4.8 Hz, H-5). The chemical shifts and the splitting pattern indicated a six- or five-membered heterocyclic ring.

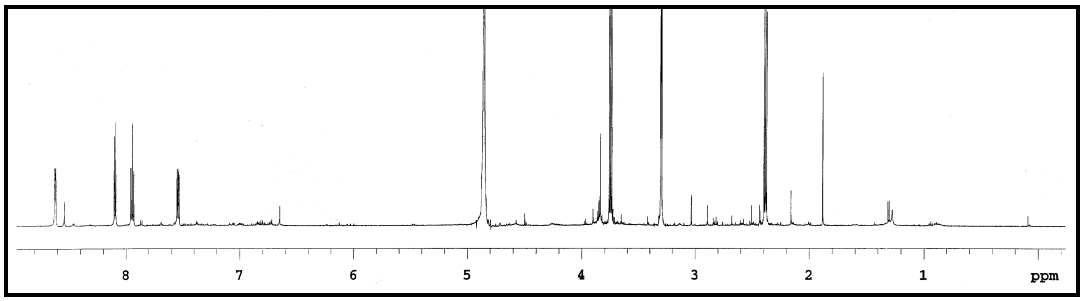


Figure 103: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of picolinamide (**76**)

The ^{13}C NMR spectrum exhibited 6 carbon signals, of which one at δ 169.3 was a carbonyl of an amide or acid. A quaternary carbon bound to a heteroatom at δ 150.9, and four sp^2 methine carbons between 149-123 suggested a six-membered ring.

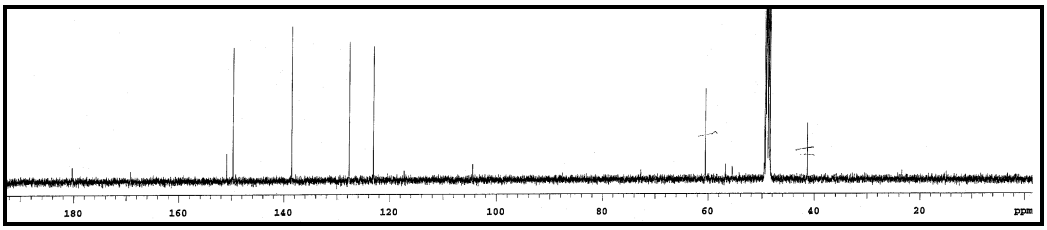


Figure 104: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of picolinamide (**76**)

The structure elucidation was completed using 2D NMR experiments. The ^{1}H,^{1}H COSY spectra displayed strong correlations between the aromatic protons, as expected from the coupling pattern for four sequential hydrogens:

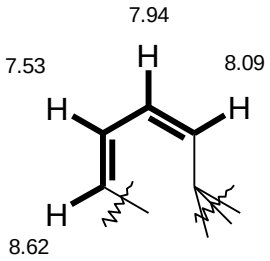


Figure 105: ^{1}H,^{1}H COSY (—) couplings of picolinamide (**76**)

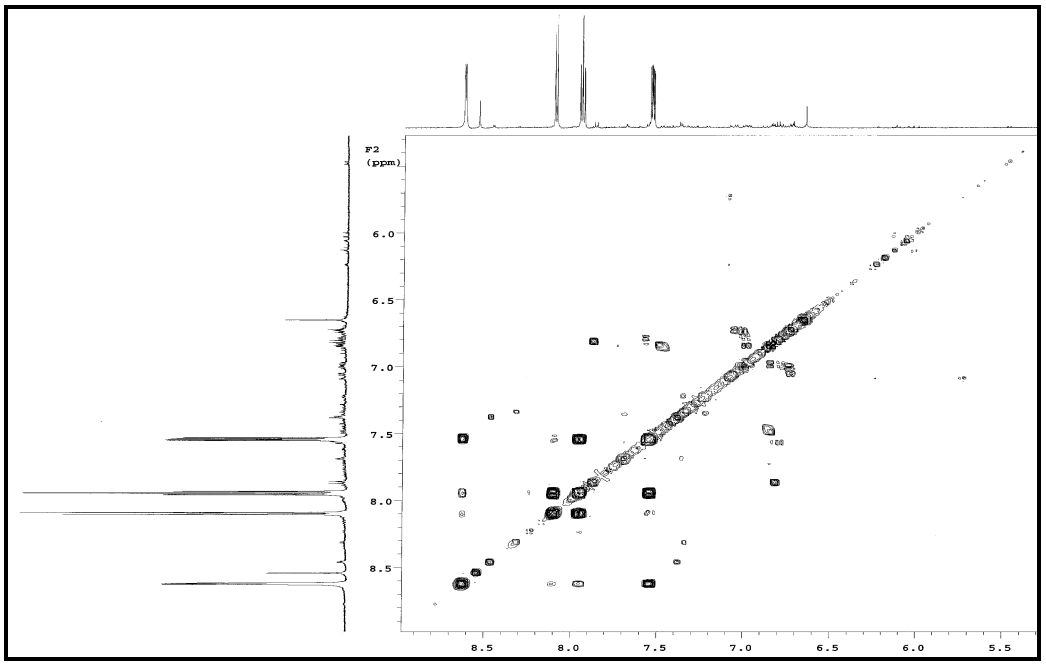
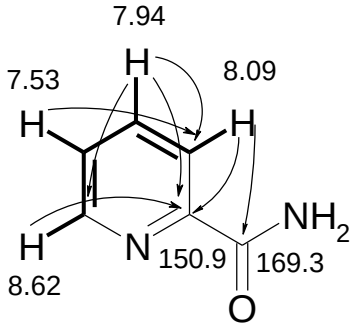


Figure 106: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of picolinamide (**76**)

The proton coupling pattern together with the formula are only in agreement for a 2-substituted pyridine, i.e. picolinamide (**76**)**.** Correspondingly, the doublet at δ 8.09 displayed a ^{3}J HMBC coupling to the carbonyl at δ 169.3 to confirm the position of the carboxamide at position 2 of the pyridine. A strong correlation from the proton at δ 7.94 to the quaternary carbon at δ 150.9 that also showed week correlation from the proton at 8.62 adjacent to the nitrogen atom was also seen:



76

Figure 107: Selected ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of picolinamide (**76**)

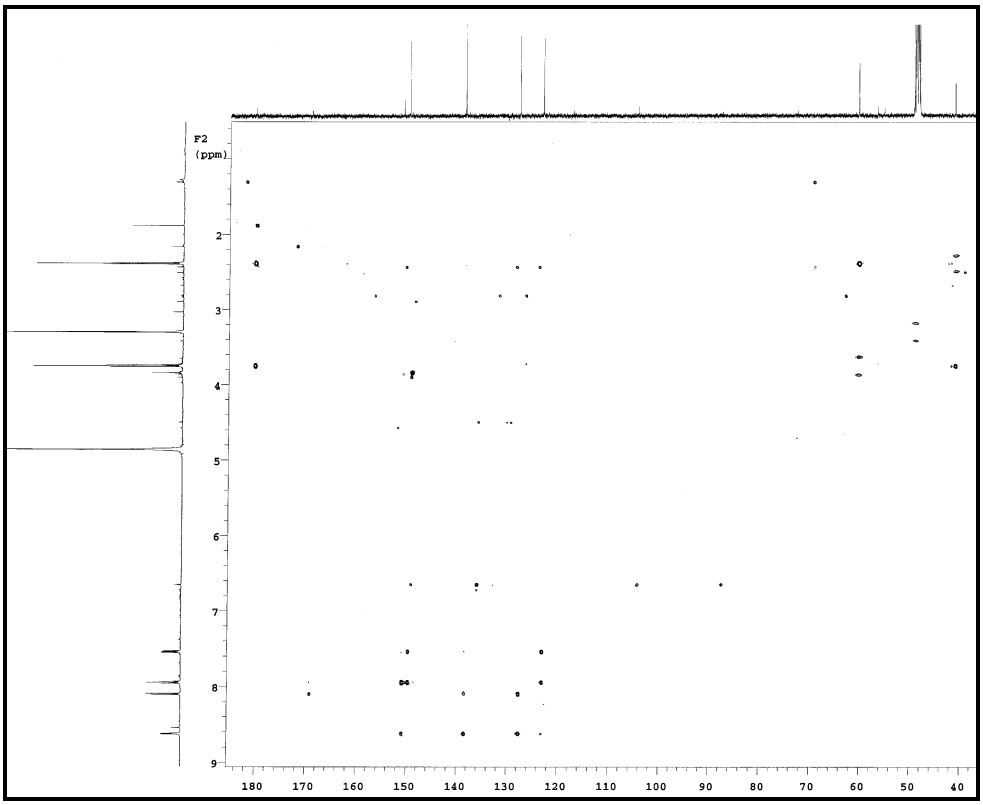


Figure 108: HMBC spectrum (CD_3OD, 500 MHz) of picolinamide (**76**)

A search in AntiBase supported by mass, ^{1}H, ^{13}C NMR and 2D data gave no results, which confirmed that picolinamide (**76**) was new as microbial natural product. Picolinamide (**76**) was found as a strong inhibitor of poly (ADP-ribose) synthetase of nuclei from rat pancreatic islet cells [117,118].

4.7 Terrestrial *Streptomyces* sp. ADM 9

The crude extract of the terrestrial *Streptomyces* sp. ADM 9 showed antimicrobial activity as mentioned in Figure 252. The TLC analysis exhibited different coloured zones (blue, red and yellow) with anisaldehyde/sulphuric acid and Ehrlich's reagent, which indicated the presence of indole derivatives.

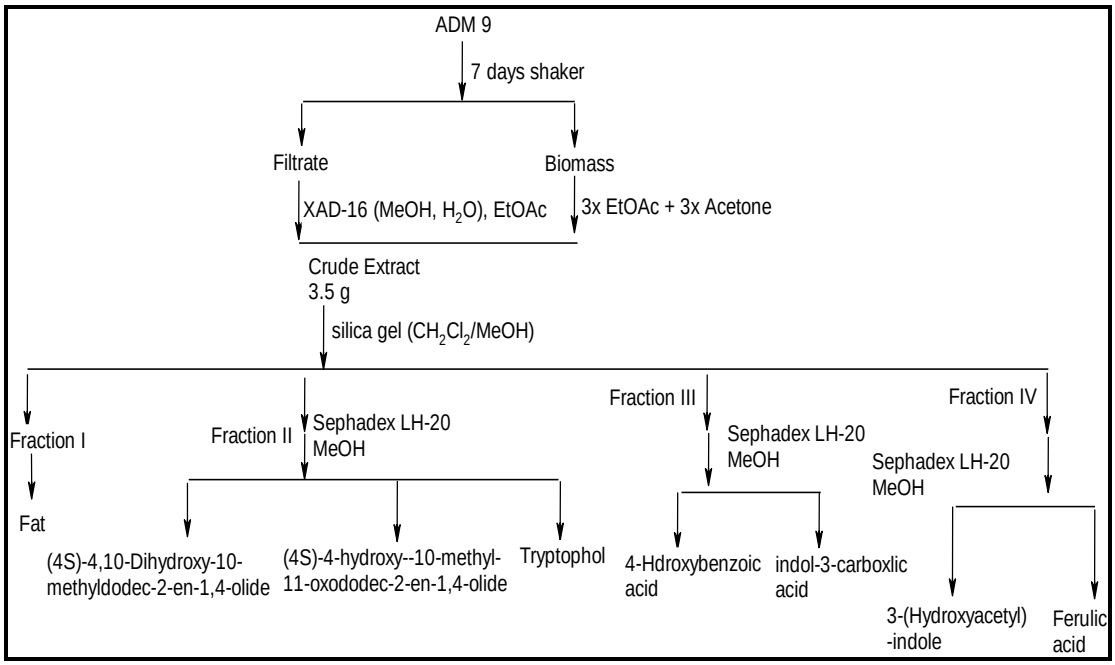
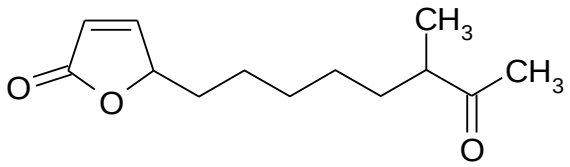


Figure 109: Work-up scheme for the terrestrial *Streptomyces* sp. ADM 9

4.7.1 4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide

Compound **77** was isolated as colourless, UV-inactive oil from fraction II in the non-polar region and turned to violet and later red by anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **77** displayed three signals at δ 7.70 (dd), δ 6.11 (dd) and δ 5.12 (m), which were typical for a butenolide moiety. In the aliphatic region, one methyl doublet at δ 1.06, one methyl singlet at δ 2.13 and the multiplet of five methylene groups between δ 1.20 and 1.70 were observed. ESIMS showed a *pseudo*molecular ion peak at *m/z* 247 that resulted in a molecular formula $C_{13}H_{20}O_3$ by HRESIMS**.** A search in AntiBase[77] by using the spectroscopic data and the molecular formula and comparing with literature data[119] and authentic spectra led to the assignment of the isolated compound as 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**77**), which was isolated previously for the first time in our group from the *Streptomycete* strains B 5632 and B 3497 from marine sediments.[119]



77

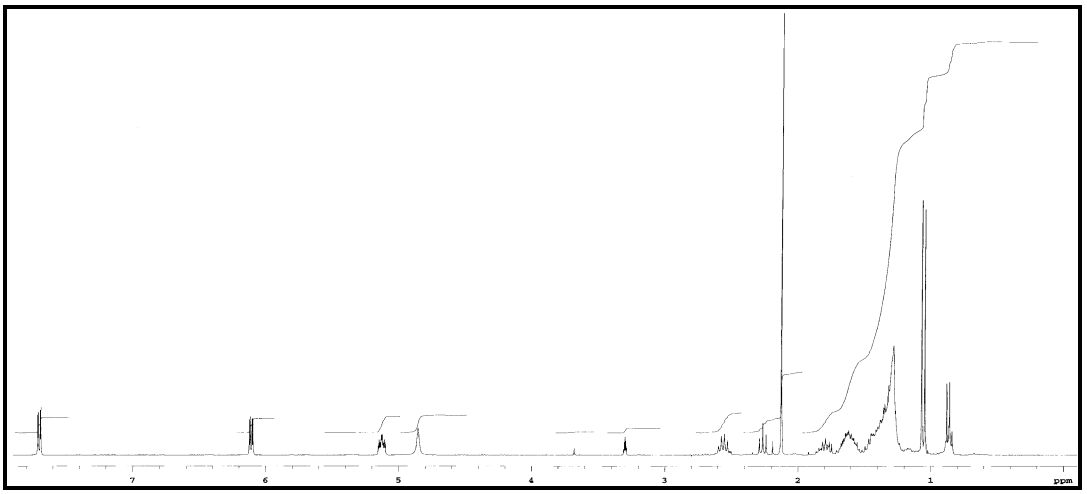


Figure 110: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**77**)

4.7.2 4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide

Subfraction II showed no UV absorbing bands in the nonpolar region but turned to violet with anisaldehyde/sulphuric acid and heating. Two compounds were isolated as colourless oils from fraction II by elution from a silica gel column followed by RP-18 column separation. The ^{1}H NMR spectrum of **78** exhibited in the olefinic region 1H doublets at δ 7.70, 6.11 (dd) and 5.13 (m), which are again typical for a butenolide moiety. In the aliphatic region, one methyl triplet at δ 0.87, one methyl singlet at 1.09 and the multiplet of six methylene groups between 1.20 and 1.80 were observed.

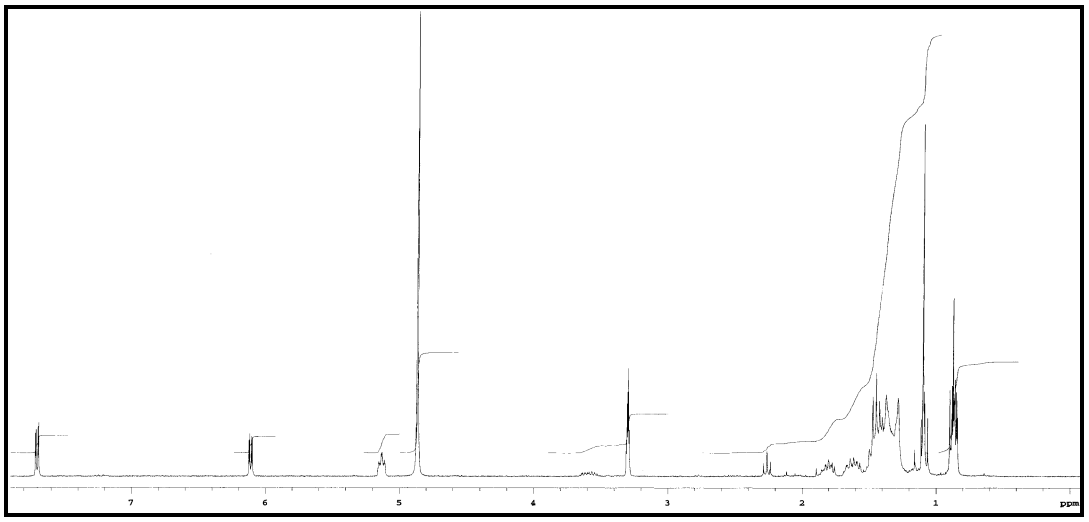
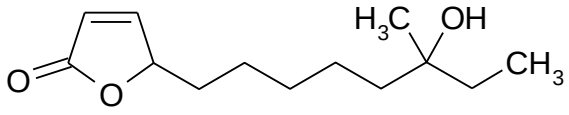


Figure 111: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 4,10-dihydroxy-10-methyldodec-2-en-1,4-olide (**78**).

ESIMS of **78** showed a *pseudo*molecular ion peak at *m/z* 226. A search in Anti-Base using the above spectroscopic data as well as the molecular weight and comparing with literature data[119] assigned the isolated compound as 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**78**). Butanolides, a family of *α,β*-unsaturated lactones, are widespread among bacteria [85] fungi [120] and gorgonians.[121]



78

4.7.3 Ferulic acid

Ferulic acid (**79**) was isolated as a colourless powder from subfraction IIIa. The ^{1}H NMR spectrum showed in the aromatic region two doublets at δ 7.55 (J = 15.9) and 6.30 (J = 15.9) of an *α,β*-unsaturated acid derivative. The coupling constant was very high indicative of a *trans* double bond. In addition, three protons at δ 7.16 (J = 1.9), 7.04 (dd, J = 2.1, 8.2), 6.79 (J = 8.2) indicated a 1,2,4-trisubstituted benzene ring. In the aliphatic region, only a methoxy signal at δ 3.88 was observed.

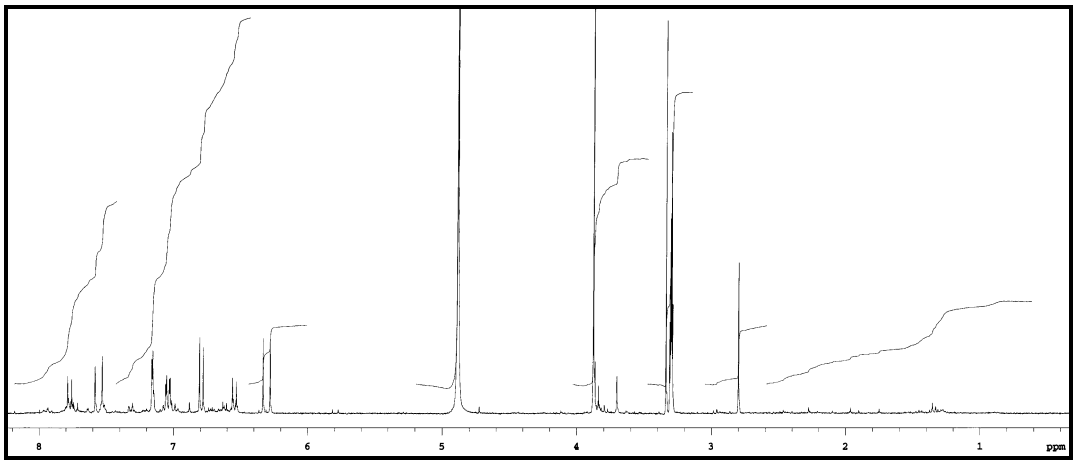
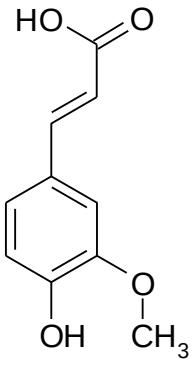


Figure 112: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of ferulic acid (**79**)

By a search in AntiBase using the spectroscopic data above and by comparison with literature data and authentic spectra, the isolated compound was assigned as ferulic acid (**79**)**,** which is widespread in plants,[122, 123] but frequently also found in bacteria.



79

4.8 Terrestrial *Streptomyces* sp. ANK 179

The terrestrial *Streptomyces* sp. ANK 179 was selected according to the chemical and biological screening. The TLC analysis exhibited different coloured zones with anisaldehyde/sulphuric acid and the crude extract showed good biological activities on agar against different microorganisms as shown in Figure 253.

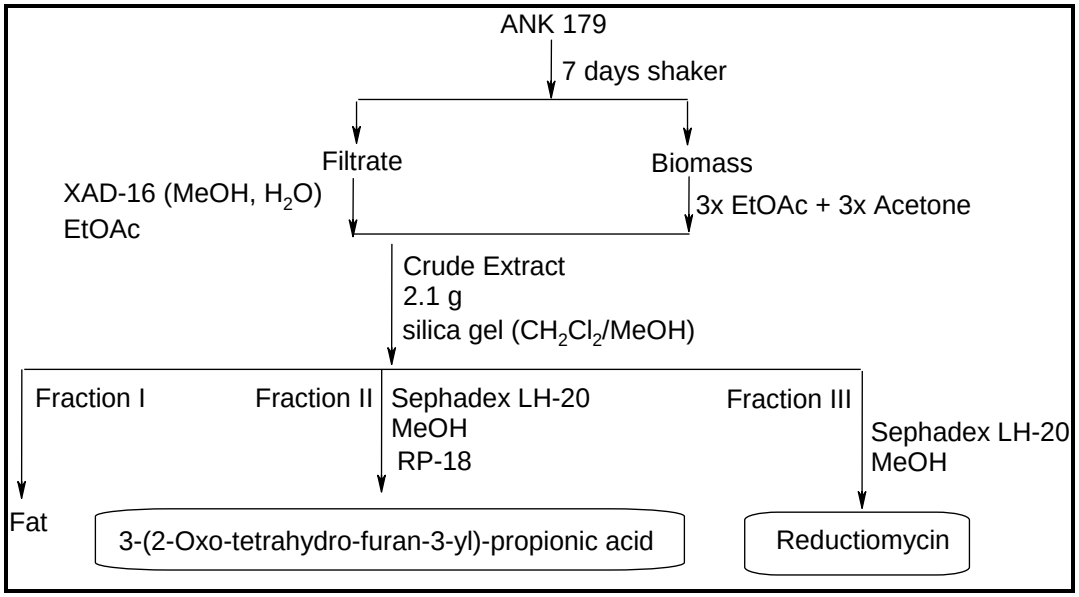


Figure 113: Work-up scheme for the terrestrial *Streptomyces* sp. Ank 179

4.8.1 Reductiomycin

Reductiomycin (**80**) was isolated as yellow needles from fraction III. It showed UV activity and turned dark green on heating after spraying with anisaldehyde reagent. The ^{1}H NMR spectrum showed a broad singlet of a H/D exchangeable proton at

δ 13.91. In the aromatic region another broad NH singlet was seen at δ 8.05 and furthermore two doublets at δ 7.49 (J = 15.0) and 5.9 (J = 15.0) were visible. The coupling pattern and the coupling constants indicated a *trans* disubstituted double bond of an α,β-unsaturated carbonyl of acid, ester or amide. In addition, a 1H singlet at δ 6.86 was exhibited, as well as a doublet of doublet at δ 6.73 (^3J = 7.5, ^4J = 2.3) for either an sp^2 attached proton or methine connected with two heteroatoms. In the aliphatic region, the spectrum showed a doublet of doublet with integration for 1H at δ 3.03 (^4J = 1.4, ^3J = 7.5) and 5 proton signals overlapped in the region of δ 2.75-2.50. Finally one methyl singlet at δ 2.12 attached to an sp^2 carbon e.g. as an acetyl group was observed.

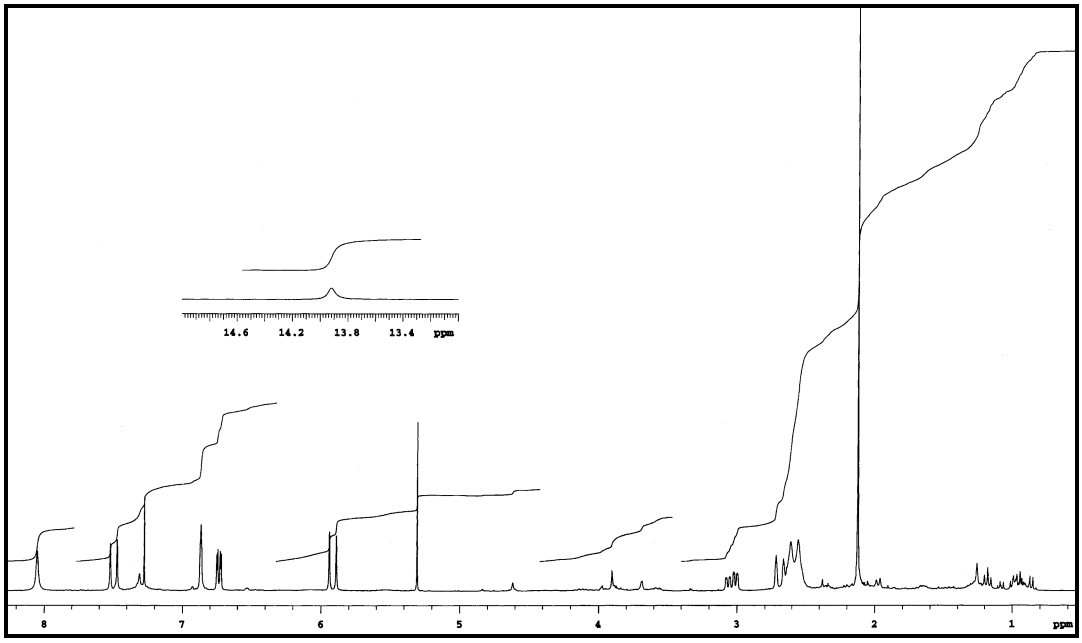
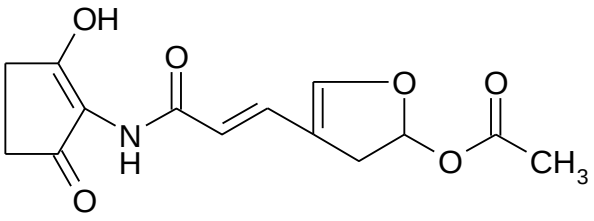


Figure 114: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of reductiomycin (**80**)

The odd molecular weight of *m/z* 293 determined by EIMS indicated the presence of an odd number of nitrogen atoms. A search in AntiBase supported by ^{1}H and MS data led to reductiomycin (**80**). It was further confirmed by the literature data and comparison with authentic spectra. Reductiomycin (**80**) showed high activity against Gram-positive bacteria and against *Trichophyton mentagrophytes* and *Alternaria solani*, also at low concentration. The acute toxicity (LD_{50}) of reductiomycin (**80**) by the intraperitoneal route in mice was about 80 µg/kg.[124]



80

4.8.2 3-(2-Oxo-tetrahydrofuran-3-yl)-propionic acid

Compound **81** was isolated as a colourless non-UV absorbing oil with medium polarity, which turned violet with anisaldehyde/sulphuric acid on TLC. ESIMS delivered a molecular weight of *m/z* 158, and HRESIMS established the molecular formula as $C_7H_{10}O_4$. The ^1H and ^{13}C NMR spectra showed an oxy-methylene group (δ_H 4.35 ddd, 4.20 m; δ_C 68.2) and one methine multiplet (δ_H 2.68; δ_C 39.8). Additionally, one methylene triplet was observed at δ 2.44 (J = 7.6 Hz). Finally, two methylene multiplets were observed in the regions of δ 2.43-1.72.

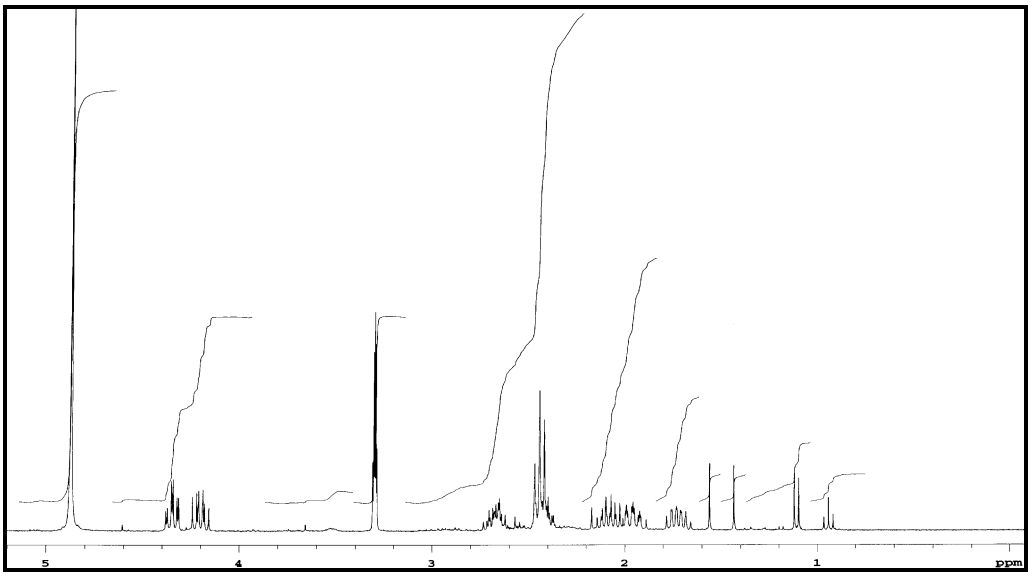


Figure 115: ^1H NMR spectrum (CD_3OD, 300 MHz) of 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**)

The ^{13}C NMR and HMQC spectra confirmed these groups and showed in addition two carbonyl signals at δ 181.7 and 177.0 of acids, esters and/or amides. Because of the three double bond equivalents and only one methine carbon, this suggested that the compound was a lactone.

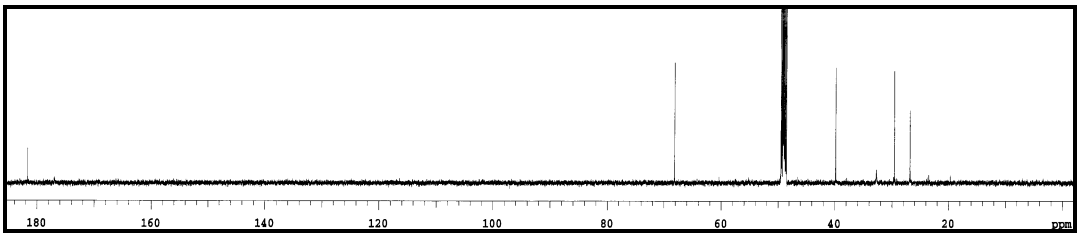


Figure 116: ^{13}C NMR spectrum (CD_3OD, 500 MHz) of 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**)

The structure was completely assigned by 2D NMR experiments. The downfield oxymethylene triplet showed COSY correlations, confirming the partial structure -OCH_2-CH_2-CH-CH_2-CH_2-.

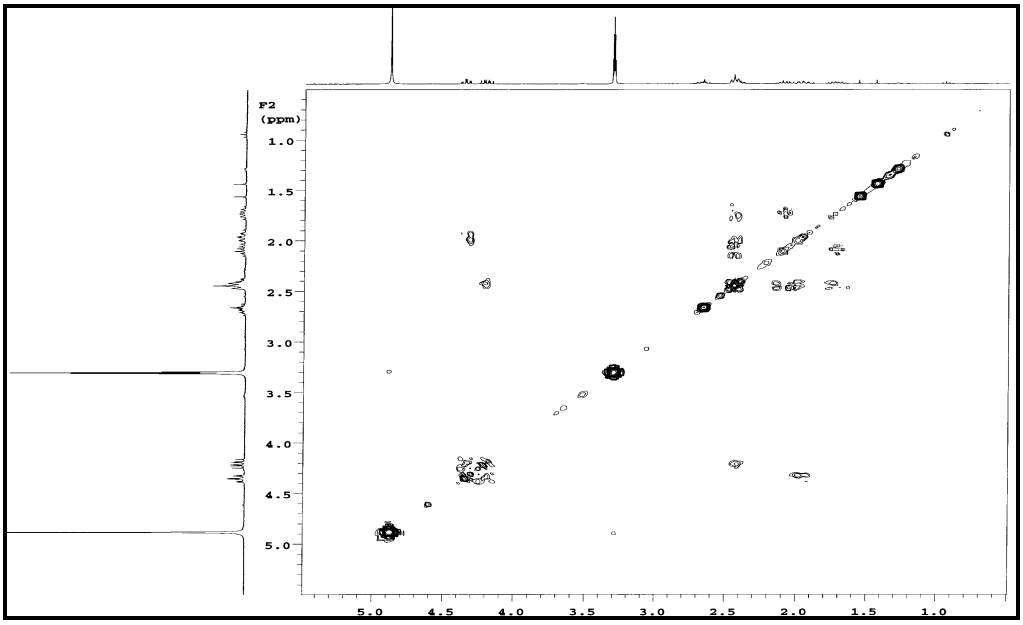


Figure 117: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 125 MHz) of 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**)

The oxymethylene group (H_2-4) further displayed a ^{3}J coupling to the carbonyl signal at δ 177.0, which showed also correlations with C-3 and C1', confirming a butanolide substituted at C-2 (δ 39.8). The chemical shifts of the methine carbon (δ 39.8) and the methylene (δ 32.7) confirmed their attachments at sp^2 carbons, which are in this case the carbonyl groups. Further correlations from H_2-2' to C-3' and C-2, from H_2-1' to C-3', C-1 and C-3, from H_2-3 to C-2, C-4, C-1' were detected. The missing correlation between the methine and the carbonyl was also observed in other γ-lactones. [125,79] This established compound as 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**).

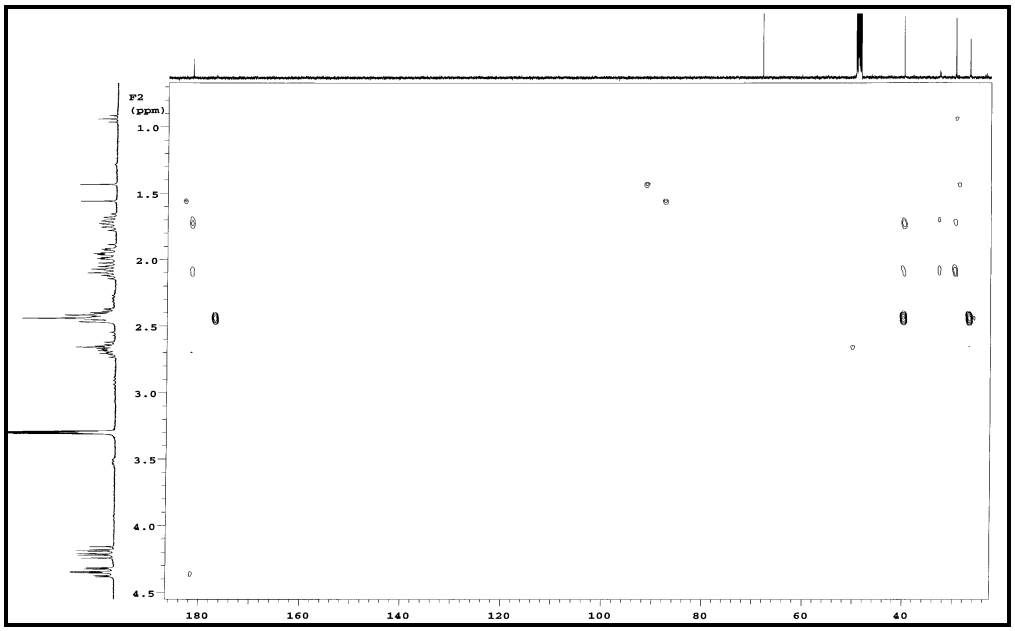
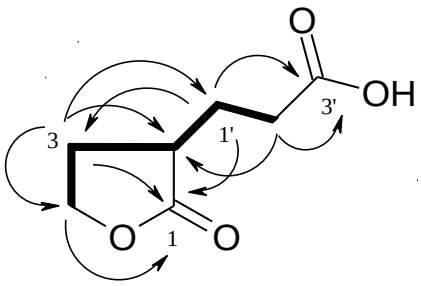


Figure 118: HMBC spectrum (CD_3OD, 500 MHz) of 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**)



81

Figure 119: $^1H,^1H$ COSY (▬) and HMBC (→) couplings of 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**)

According to the search in AntiBase[77] and the Dictionary Natural Products, 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**) is a new natural product.

4.9 Terrestrial *Streptomyces* sp. ANK 174

The terrestrial *Streptomyces* sp. ANK 174 was selected according to the chemical and biological screening. The TLC analysis exhibited different coloured zones with anisaldehyde/sulphuric acid, the crude extract showed on agar plates good biological activities against different microorganisms as shown in Figure 254.

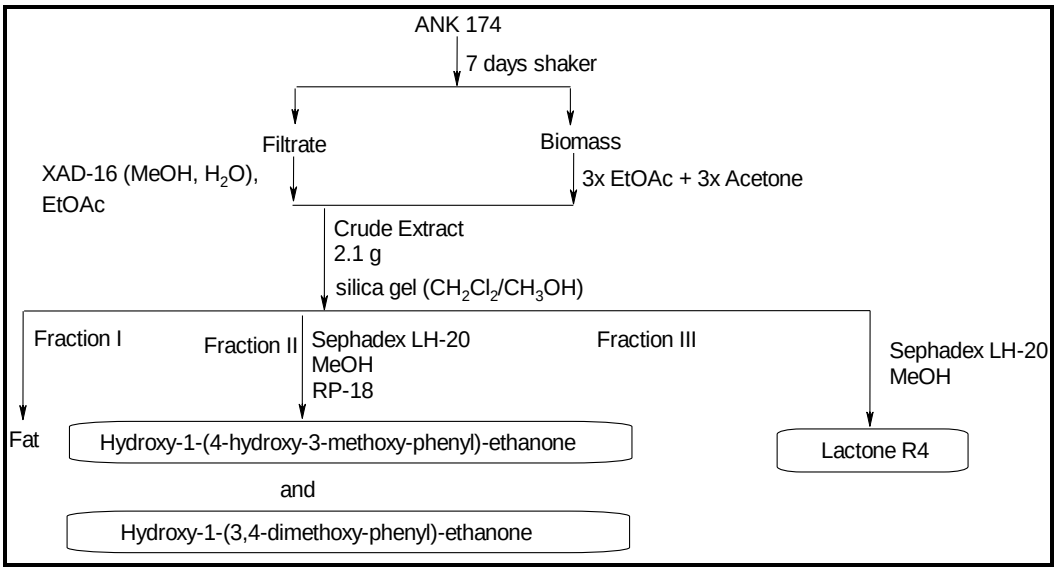


Figure 120: Work-up for scheme for terrestrial *Streptomyces* sp. Ank 174

4.9.1 Lactone R4

Lactone R4 (**82**) was isolated as colourless oily substance from a UV active fraction III, which turned to blue by spraying with anisaldehyde reagent. The ^{1}H NMR spectrum of **82** exhibited only signals in the aliphatic region. Two methine protons attached to heteroatom at δ 4.49 and 3.99 were observed, as well as methylene protons at δ 3.73 and 3.62 of an ABX system with a downfield shift indicative that they were in connection with sp^2 carbons or heteroatoms. One methine proton at δ 2.85, in addition methylene protons at δ 2.19 and 2.04 of another ABX system, and 7 overlapping proton signals in the region of 1.60-1.30 were also observed. Two overlapping proton multiplets at δ 1.14. and two overlapping methyl signals at δ 0.88 were also visible.

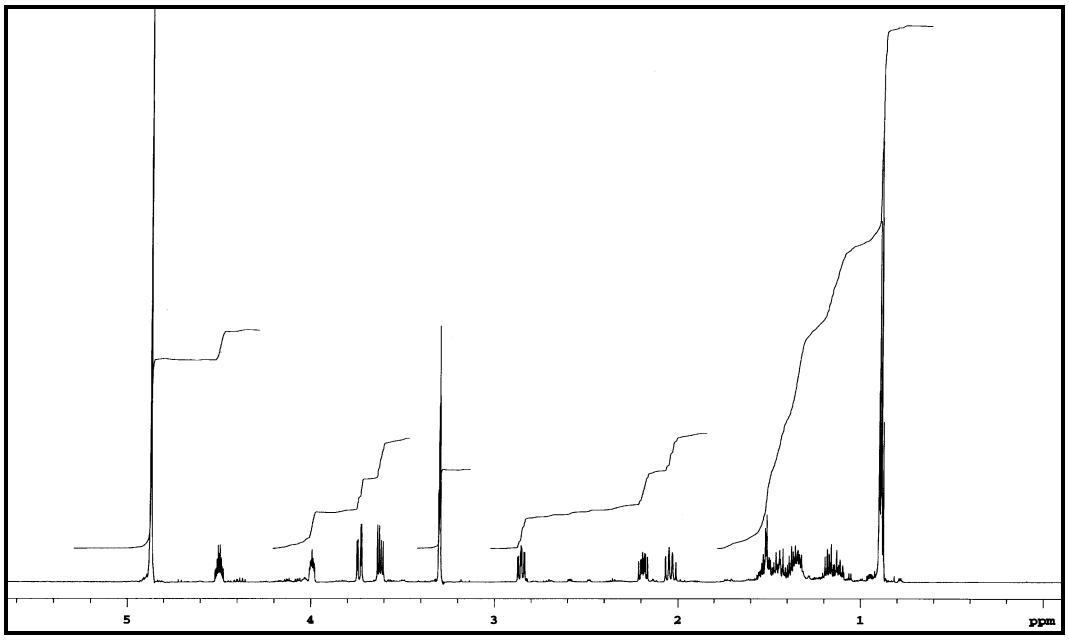


Figure 121: ^{1}H NMR spectrum (CD_3OD, 125 MHz) of lactone R4 (**82**)

The ^{13}C NMR and HSQC data showed 12 carbon signals. A carbonyl signal of an acid derivative at δ 180.1, two oxymethine carbons at δ 81.0, 70.4 and an oxymethylene group at δ 64.9 were observed. In addition to two methines at δ 47.8 and 33.8, four methylene groups at δ 35.7, 34.2, 30.5 and 24.3 as well as two methyl groups at δ 19.6, 11.7 were present.

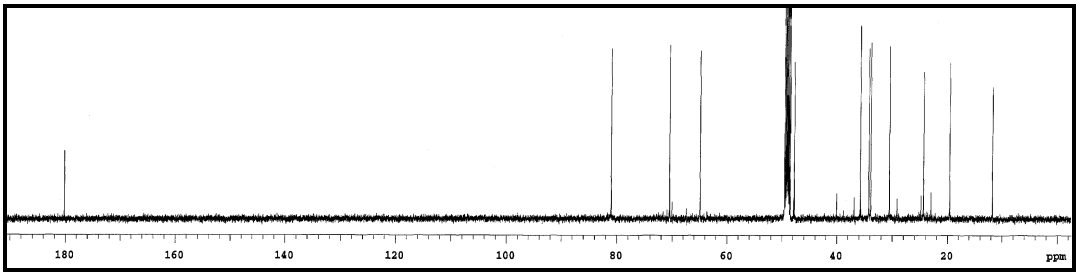


Figure 122: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of lactone R4 (**82**)

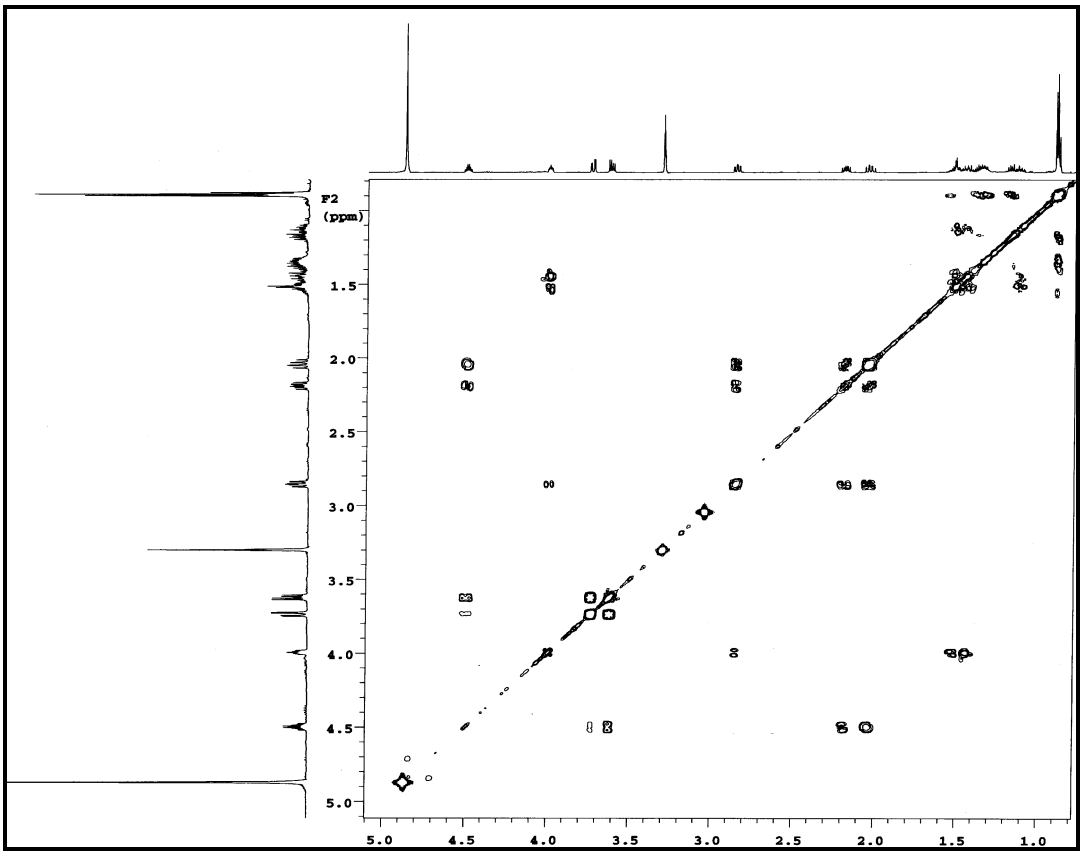


Figure 123: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 125 MHz) of lactone R4 (**82**)

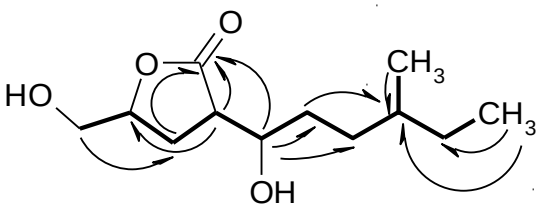
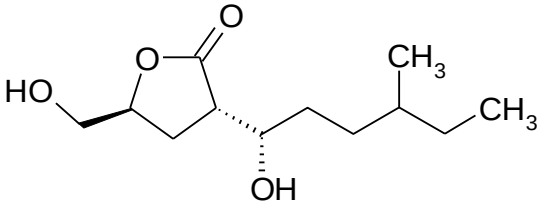


Figure 124: $^{1}H,^{1}H$ COSY (▬) and HMBC (→) couplings of lactone R4 (**82**)

A search in AntiBase[77] with the ^{1}H NMR, ^{13}C NMR and 2D data gave lactone R4 (**82**) as a result. This was further confirmed by the literature data[126] and comparing with authentic spectra. It was isolated previously from *Streptomyces* sp. GT 061089.[127]



82

4.9.2 Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone

Compound **84** was isolated as colourless solid, which changed to greenish blue on spraying with anisaldehyde reagent and heating. The ^{1}H NMR spectrum of **84** in CD_3OD exhibited 3 proton signals in the aromatic region: two *ortho*-coupled protons at δ 7.50 (dd, J = 8.2, 2.0 Hz) and 6.85 (d, J = 8.2 Hz) and a *meta* coupled proton at δ 7.52 (d, J = 2.0 Hz). The coupling pattern and the coupling constants indicated a 1,2,4-tri-substituted benzene ring. In the high field region, two oxygenated groups, one 2H signal of an oxy-methylene group at δ 4.83, and the 3H signal of a methoxy group at δ 3.90 were displayed.

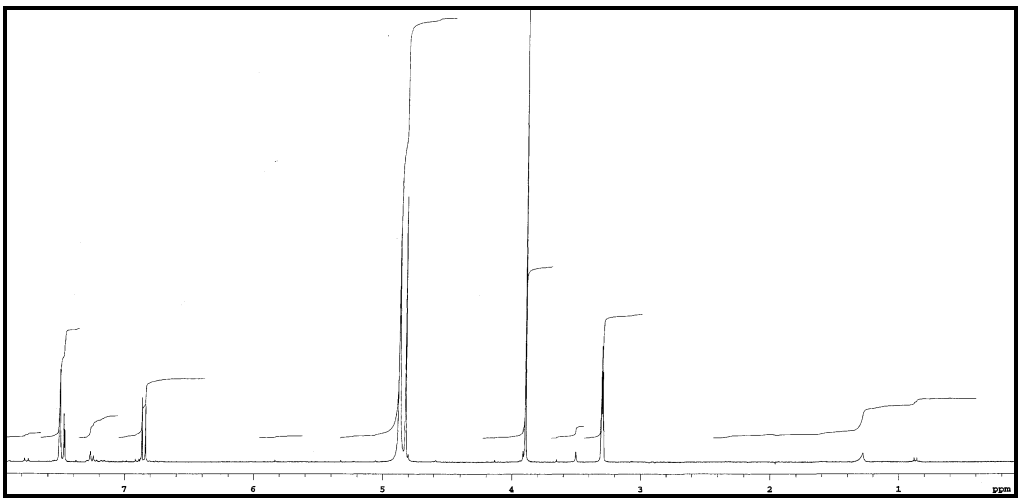


Figure 125: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of hydroxy-1-(4-hydroxy-3-methoxyphenyl)-ethanone (**84**)

The ^{13}C NMR and HMQC spectra indicated the presence of 9 carbon signals: Of these one was a carbonyl group at δ 198.5, six were sp^2 carbons of a benzene ring. Two oxygenated carbons appeared downfield at δ 149.5 (C-3) and δ 154.6 (C-4); three methine carbons C-2, C-5 and C-6 were seen at δ 111.4, 116.2 and 124.0, respectively, and the last carbon C-1 was found at δ 127.2. Additionally, in the sp^3 region, signals of oxy-methylene and oxy-methyl groups at δ 65.8 and 56.4 were displayed.

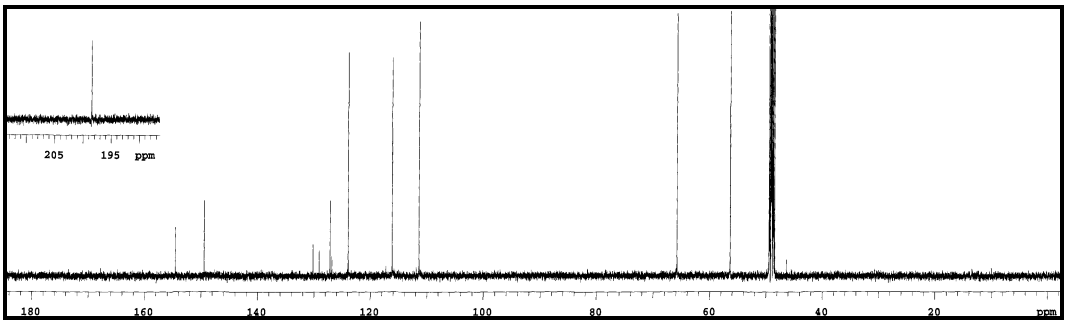
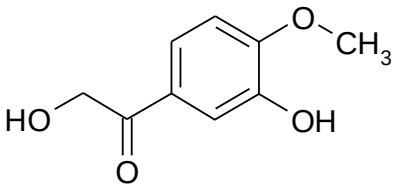
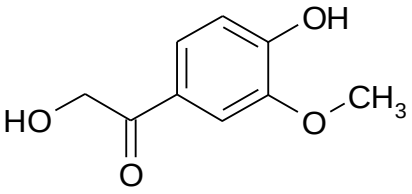


Figure 126: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**)

From the spectral data, there are two structure possibilities **83, 84**



83



84

Subjecting the compound **84** to HMBC measurement, a ^3J correlation from the methoxy group (δ 3.90) to C-3 (δ 149.5) and also a ^3J correlation from the doublet of C-5 (δ_H 6.85) to C-3 (δ 149.5) were observed. Structure **83** was therefore excluded because there was a ^3J correlation from H-2 (δ_H 7.52) and H-6 (δ_H 7.50) with the carbonyl carbon (δ_C 198.5). Furthermore, a correlation was observed from the methylene protons to the carbonyl carbon. This finally confirmed the structure as 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**).

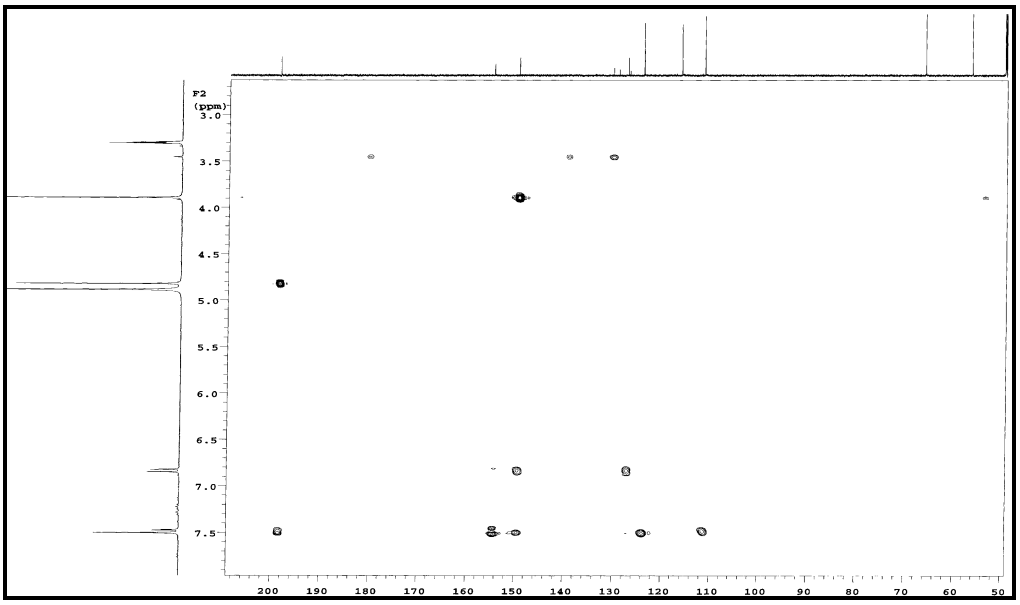
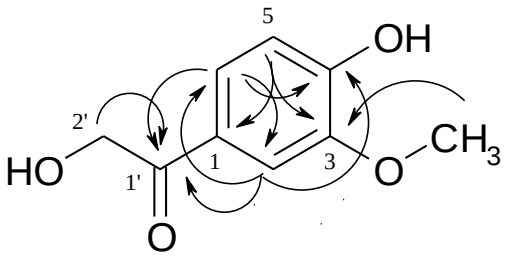


Figure 127: HMBC spectrum (CD_3OD, 125 MHz) of hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**)



84

Figure 128: Selected HMBC (→) correlations of 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**)

Table 13: ^{1}H and ^{13}C NMR assignments of compound **84** (*J* in [Hz]).

Position	δ_C [a,b]	δ_H [a,c]
1	127.2, C_q	-
2	111.4, CH	7.52 (d, 2.0)
3	149.5, C_q	-
3-OCH_3	56.4, CH_3	3.90 (s)
4	154.6, C_q	-
5	116.2, CH	6.85 (d, 8.2)
6	124.0, CH	7.50 (dd, 8.2, 2.0)
1'	198.5, C_q	-
2'	65.8, CH_2	4.83 (s)

[a] CD_3OD; [b] (150 MHz); [c] (300 MHz)

4.9.3 2-Hydroxy-1-(3,4-dimethoxy-phenyl)-ethanone

2-Hydroxy-1-(3,4-dimethoxy-phenyl)-ethanone (**85**) had a lower polarity than the pervious compound **84** and was obtained as colourless solid, showing the same colour reaction as the acetophenone derivative **84**. The molecular weight was deduced by EI MS as *m/z* 196, and HRESIMS resulted in the molecular formula $C_{10}H_{12}O_4$ with the difference of Δm = 14 amu between **85** and **84**. The ^{1}H and ^{13}C NMR spectra of **85** displayed a similar pattern as **84**, except in the up field region, where an additional methoxy group at δ 3.90 (δ_C 56.5) was found. The OH group of compound **84** was obviously methylated in **85**. A complete 2D NMR correlation confirmed the structure as 1-(3,4-dimethoxy-phenyl)-2-hydroxy-ethanone (**85**).

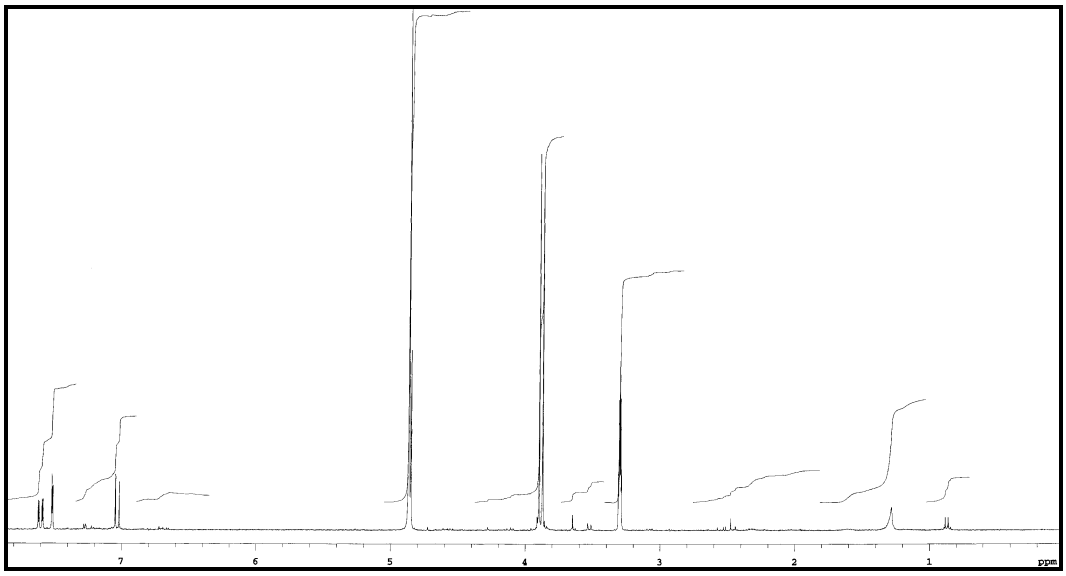


Figure 129: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 1-(3,4-dimethoxy-phenyl)-2-hydroxy-ethanone (**85**).

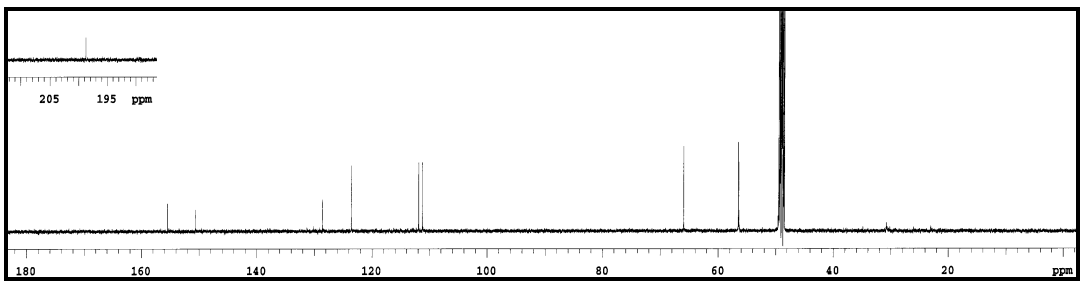
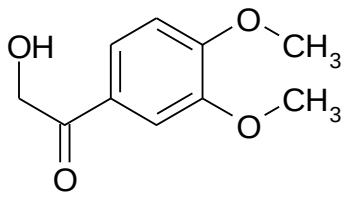


Figure 130: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 1-(3,4-dimethoxy-phenyl)-2-hydroxy-ethanone (**85**).



85

Table 14: ^{1}H and ^{13}C NMR assignments of compound **85** (*J* in [Hz]).

Position	δ_C [a,b], mult.	δ_H [a,c], mult.
1	128.6, C_q	-
2	111.3, CH	7.52 (d, 2.0)
3	150.7, C_q	-
3-OCH_3	56.45, CH_3	3.87 (s)
4	155.5, C_q	-
4-OCH_3	56.51, CH_3	3.90 (s)
5	111.9, CH	7.03 (d, 8.4)
6	123.6, CH	7.60 (dd, 8.4, 2.0)
1'	198.8, C_q	-
2'	66.0, CH_2	4.85 (s)

[a] CD_3OD; [b] 125 MHz; [c] 300 MHz

4.10 Terrestrial *Streptomyces* sp. WO 990

The crude extract of the terrestrial *Streptomyces* sp. WO 990 showed strong biological activity against a variety of microorganisms, Figure 255, and the TLC analysis exhibited two UV active zones, which turned to red with anisaldehyde/sulphuric acid. Ehrlich's reagent indicated the presence of indole derivatives.

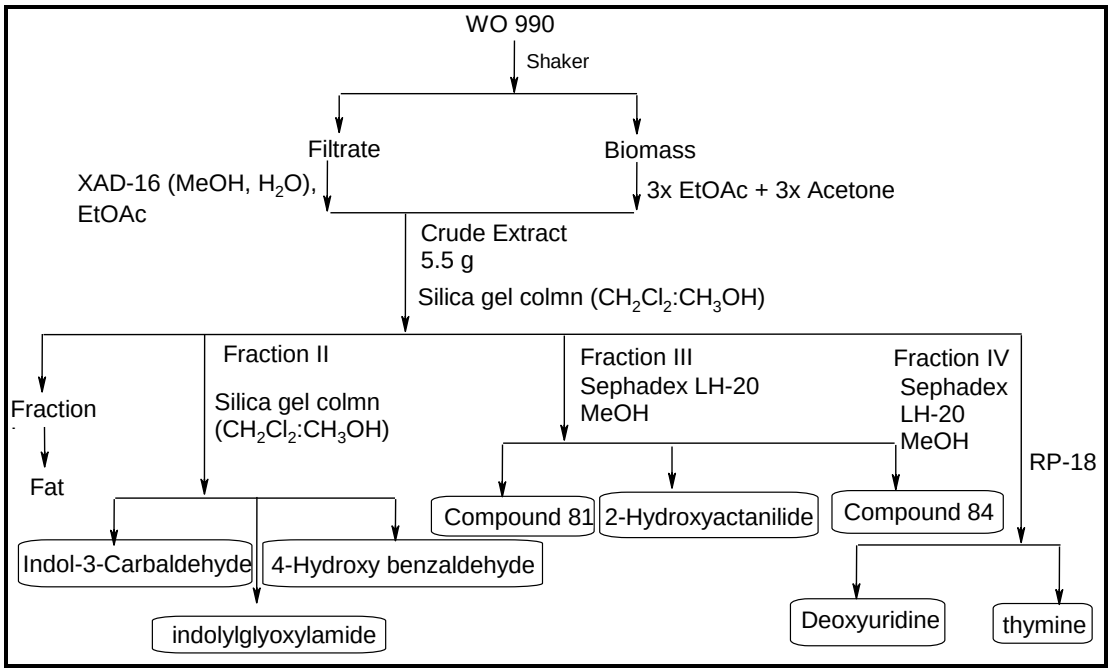


Figure 131: Work-up scheme for terrestrial *Streptomyces* sp. WO 990

4.10.1 1-(4-Hydroxy-phenyl)-butane-2,3-diol

1-(4-Hydroxy-phenyl)-butane-2,3-diol (**86**) was isolated from fraction III as reddish oily substance. It showed an absorbing band at 254 nm and turned red on spraying with anisaldehyde reagent and heating. The ^{1}H NMR spectrum exhibited in the aromatic region two 2H *ortho* coupled signals at δ 7.05 and 6.68 (J = 8.5), which were indicative for a 1,4-disubstituted aromatic ring. The upfield shift of these signals gave evidence for electron donating groups on the aromatic ring. In the aliphatic region there were two methines attached to heteroatoms at δ 3.61 and 3.49. Additionally one methylene group with protons at δ 2.76 and 2.55 appeared as an ABX system due to the presence of a chiral centre. Finally a terminal methyl doublet at δ 1.16 was observed.

ESI MS afforded *pseudo*molecular ions at 205 *m/z* $[M+Na]^+$ and 181 $[M-H]^-$, which delivered the molecular weight as 182 Dalton. HRESIMS revealed the molecular formula $C_{10}H_{14}O_3$.

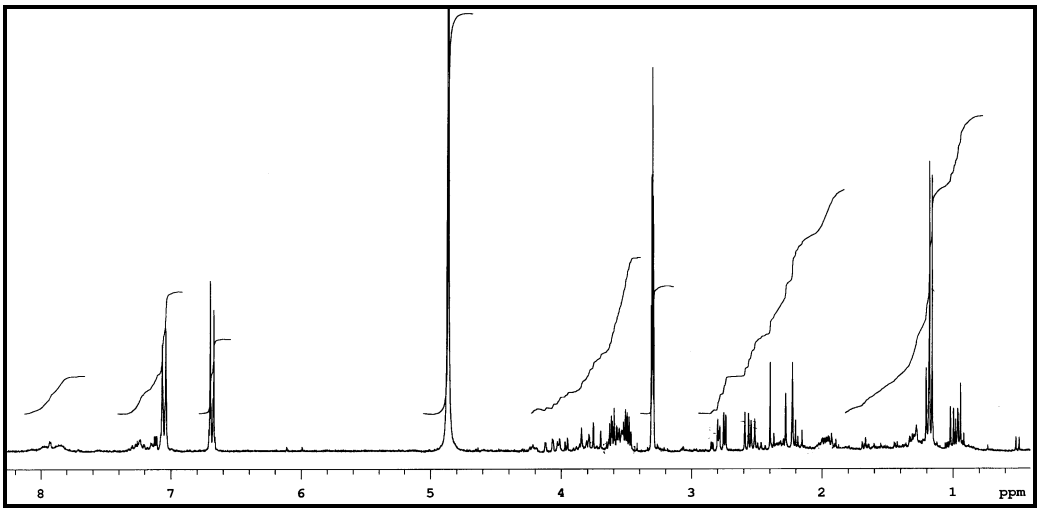


Figure 132: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

The ^{13}C NMR and HSQC spectra of **86** showed 7 carbon signals: one sp^2 oxycarbon at δ 156.7, and two methine sp^2 carbons at δ 131.4 and 116.0 with an intensity of each 2H were observed. In addition an overlapping quaternary carbon signal at δ 131.4 was also present. In the aliphatic region, two carbons at δ 77.8 and 70.4 for two hetero bound methines were visible. Moreover a methylene carbon at δ 39.4 and a methyl group at δ 19.3 were observed.

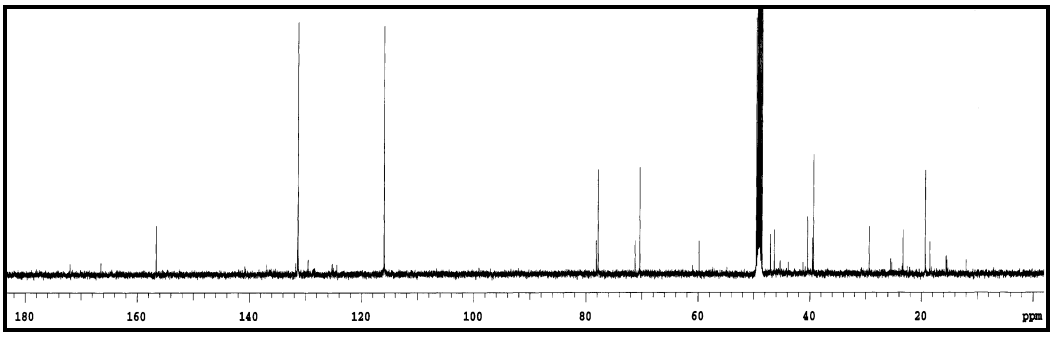


Figure 133: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

In the ^{1}H,^{1}H COSY spectrum, the doublet at δ 7.05 showed a ^{3}J correlation with the signal at δ 6.68, (Fragment **A**). Methylene signals at δ 2.76, 2.55 showed ^{3}J correlation with the oxymethine proton at δ 3.49, which itself also showed a strong ^{3}J correlation with another oxymethine proton at δ 3.61; the latter correlated with the methyl doublet at δ 1.16 (Fragment **B**).

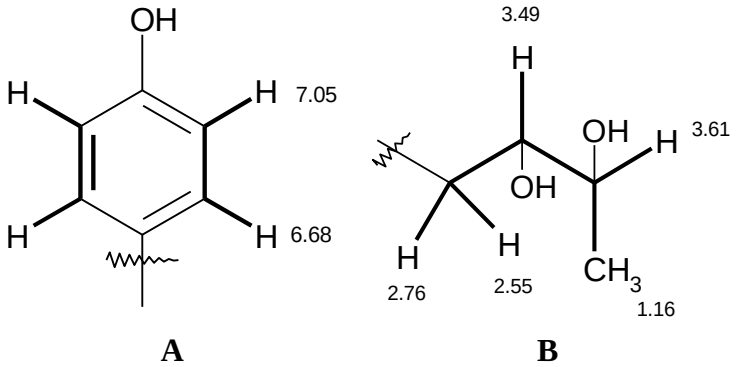


Figure 134: ^{1}H,^{1}H COSY (—) correlations in 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

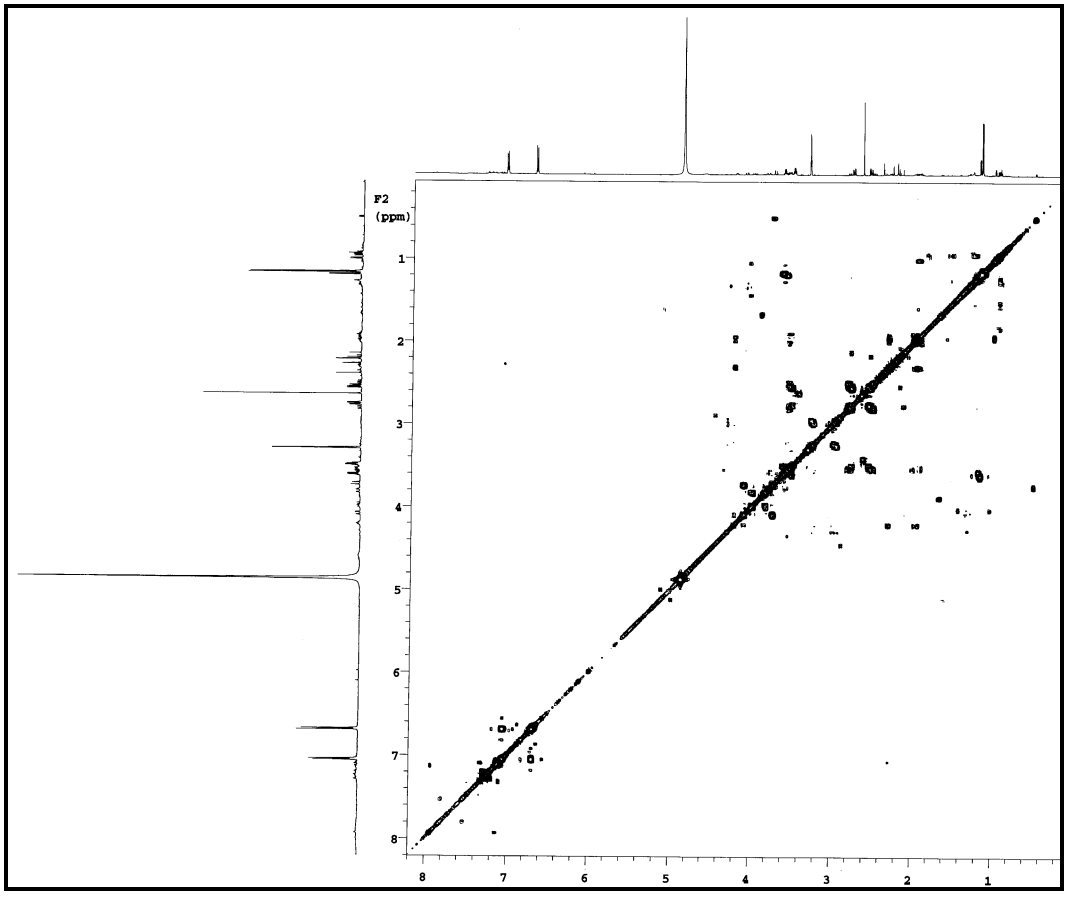
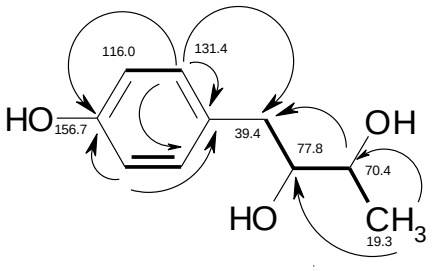


Figure 135: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

In the HMBC spectrum of **86**, the proton at δ 6.68 displayed a strong correlation with both the quaternary carbon at δ 156.7 and with another methine carbon at δ 131.4 to confirm a 1,4-disubstituted benzene ring. The methylene carbon at δ 39.4 confirmed the direct linkage between fragments **A** and **B** through the assigned quaternary carbon at δ 131.4 (C_q-1). The methyl doublet at δ 1.16 showed a ^{3}J coupling

with the oxymethine carbon at δ 77.8 and ^2J coupling with the oxymethine carbon at δ 70.4.



86

Figure 136: Selected ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

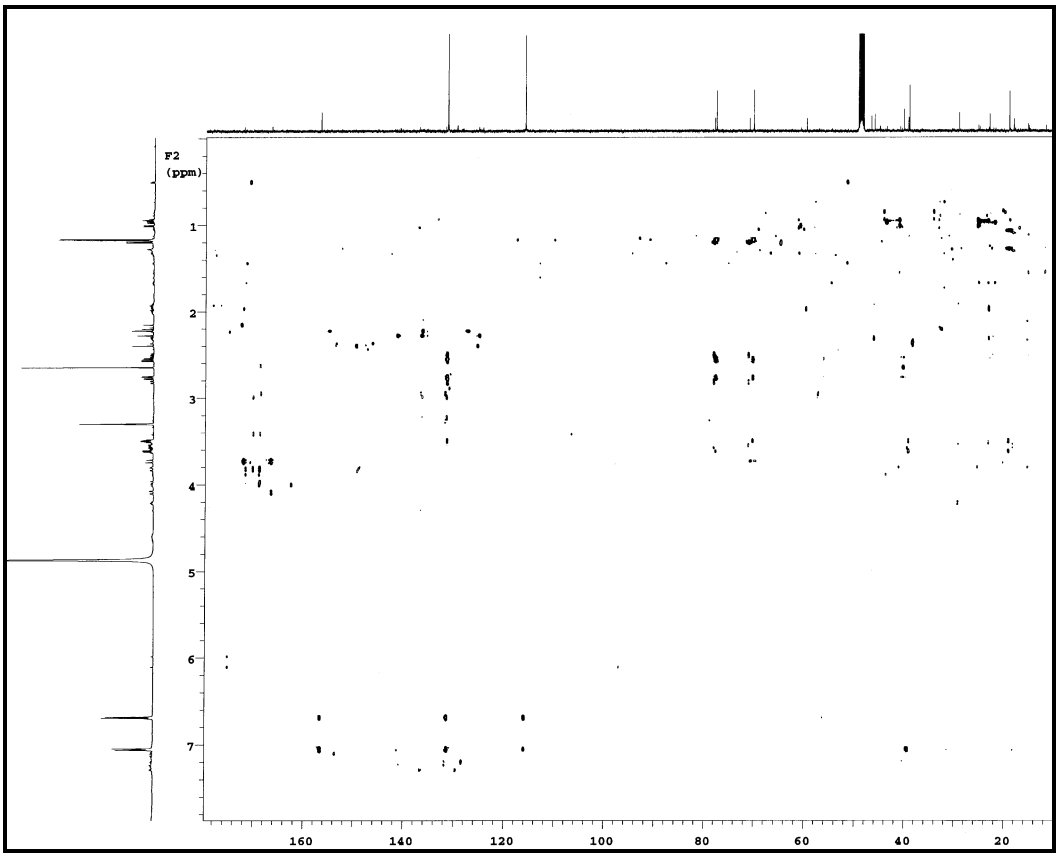
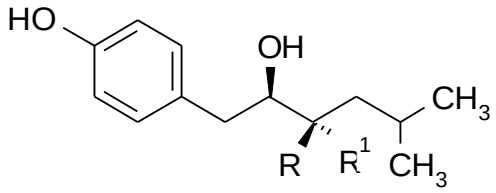


Figure 137: HMBC spectrum (CD_3OD, 125 MHz) of 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**)

A search in AntiBase[77] supported by ^{1}H, ^{13}C NMR, 2D spectroscopic data gave no result, which suggested that compound (**86**) was a new natural product. Guay-

masol (**86**) and epiguaymasol (**87**)**,** which are related triols, were isolated from a culture broth of a *Bacillus* sp. CNA-995 obtained from a deep-sea sediment core. [128]



86: R= OH, R^1= H
87: R = H, R^1= OH

4.10.2 3-Hydroxy-4-(4-hydroxy-phenyl)-butan-2-one

3-Hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**) was isolated from the same fraction as colourless oily substance, which turned red with anisaldehyde sulphuric acid spray reagent. The ESI mass spectrum showed *pseudo*molecular ions at *m/z* 203 and 383 for $[M+Na]^+$ and $[2M+Na]^+$, respectively. HRESIMS afforded the molecular formula as $C_{10}H_{12}O_3$. A difference of 2H was observed in comparison with 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**). The ^{1}H NMR spectrum exhibited in the aromatic region the same pattern and chemical shift for a 1,4-disubstituted benzene ring, pointing to a structural analogue, with the difference observed in the aliphatic region. The multiplet at δ 4.24 of a methine group attached to a heteratom was observed, and an ABX system at δ 2.93 and 2.71 indicated an attachment to an sp^2 carbon. In addition a methyl group attached to an sp^2 carbon was seen as a singlet at δ 2.10.

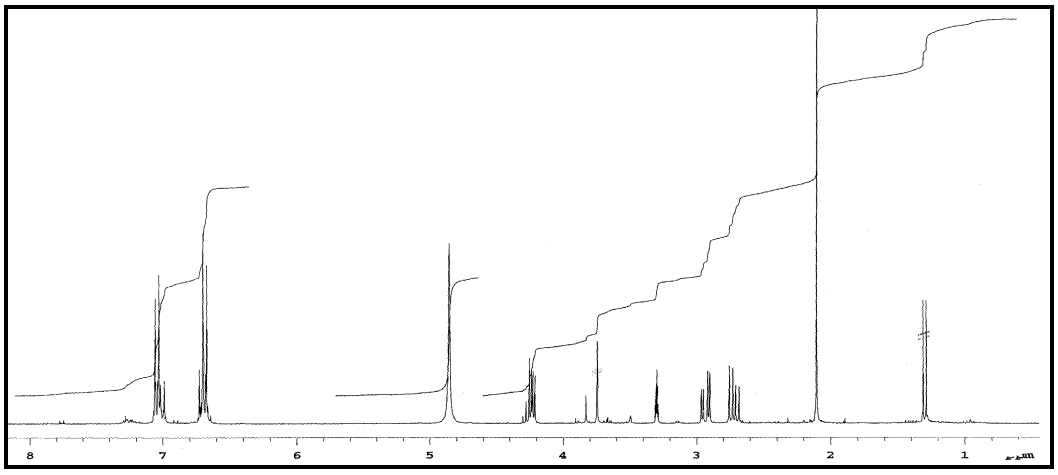


Figure 138: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**)

The ^{13}C NMR spectrum revealed 10 carbon signals, among them a ketone carbonyl at δ 213.1. In addition, 6 sp^2 carbon signals were visible: one oxymethine, a methylene group and one methyl signal.

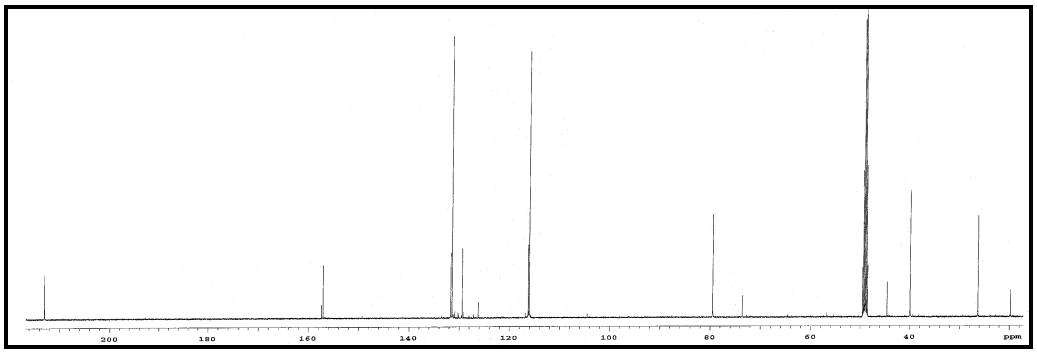
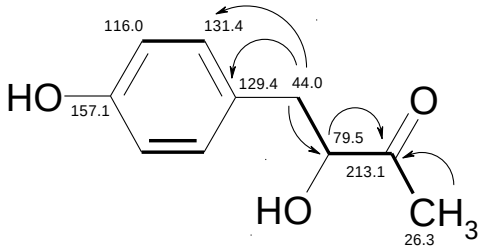


Figure 139: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**)

A search in AntiBase using the MS and NMR data yielded no result indicating a new microbial secondary metabolite. Therefore, the structure **89** was subjected to HMBC measurement which displayed long range coupling from the methyl singlet at δ 2.10 to the carbonyl at δ 213.1, which itself showed ^2J coupling with the methine multiplet at δ 4.24 and ^3J coupling with methylene group at δ 2.93, 2.71, indicating the fragment CH_3-CO-CH (OH)-CH_2. The latter methylene group displayed strong coupling with δ 131.4 and with a quaternary carbon at δ 129.4, which revealed that C-4 was connected to C-1'. The remaining hydrogens were assumed to be due to hydroxy groups at C-3 and C-4'.



89

Figure 140: $^1H,^1H$ COSY (▬) and HMBC (→) couplings of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**)

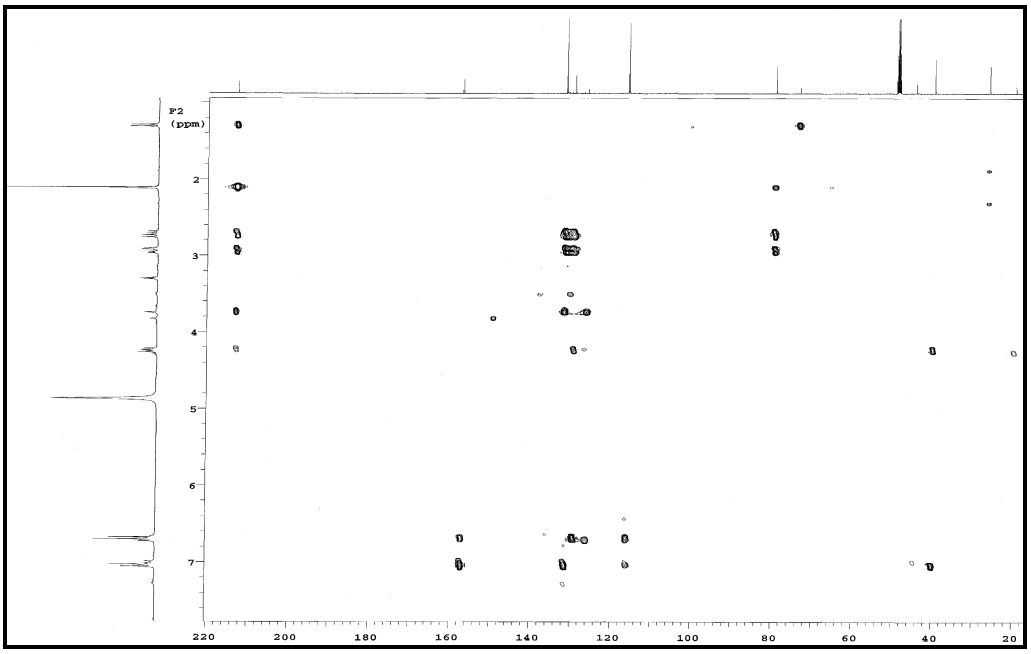


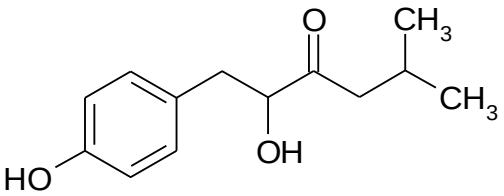
Figure 141: HMBC spectrum (CD_3OD, 125 MHz) of 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**)

A related structure is that of 4-hydroxysattabacin (**90**)**,** which was isolated from a *Bacillus* sp. strain B-60 and showed antiviral activity mainly against the HSV1 and HSV2.[129] Another related compound is kurasoin A (**91**)**,** a protein farnesyltransferase inhibitor from the culture broth of *Paecilomyces* sp. FO-36841.[130]

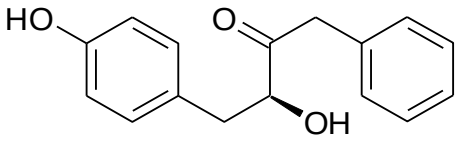
Table 15: Comparison between 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**) and 4-hydroxysattabacin (**90**)[129]

position	**89** [a,b]	**90** [c,d]
1	129.4	128.1
2	131.4	130.4
3	116.0	115.4
4	157.1	154,8
5	116.0	115.4
6	131.4	130.4
1	40.0	39.1
2'	79.5	77.6
3'	213.1	211.4
4'	26.3	47.4
5'	-	24.6
6'	-	22.6
7'	-	22.5

[a] CD_3OD; [b] 125 MHz; [c] $CDCl_3$; [d] 75 MHz



90



91

4.10.3 N-Acetyl-2-aminophenol

N-Acetyl-2-aminophenol (**92**) was isolated as a colourless solid with UV activity but no colour reaction with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **92** displayed in the aromatic region two protons doublets of doublets at δ 7.56 and 6.77. Additionally two triplets at δ 6.98 and 6.83 indicated a 1,2-disubstituted benzene; in addition, a 3H singlet at δ 2.15 indicated a methyl group connected with an sp^2 carbon.

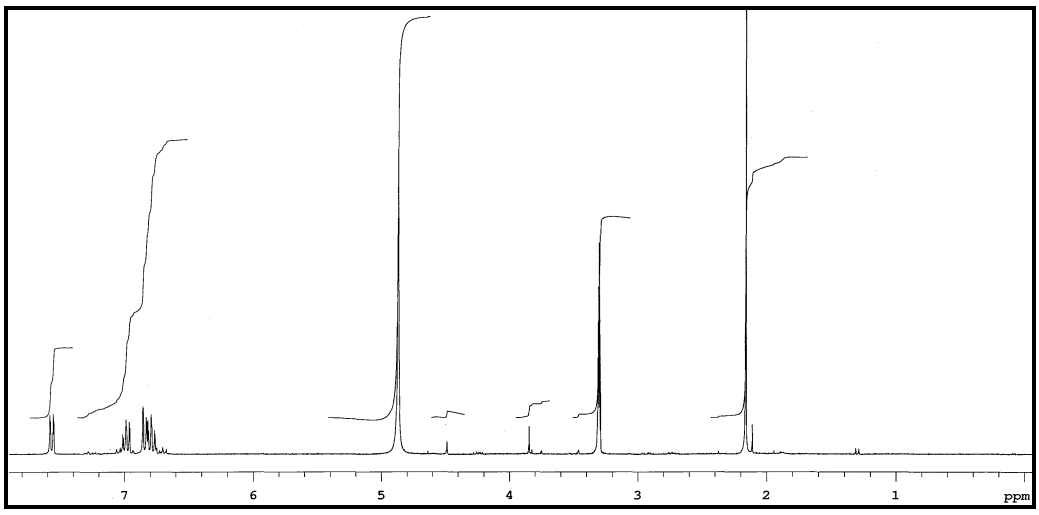
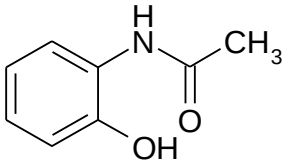


Figure 142: ^{1}H NMR spectrum (CD3OD, 300 MHz) of N-acetyl-2-aminophenol (**92**)

The molecular weight of N-acetyl-2-aminophenol (**92**) was deduced as *m/z* 151 from EIMS. Compound **92** was isolated previously from *Pseudomonas* cultures for the first time by Winkler *et al.* [131] *et al.* and in our group from a marine Streptomycete.[132]



92

4.10.4 Indolyl-3-glyoxylamide

The molecular weight of indolyl-3-glyoxylamide (**93**) was deduced as *m/z* 188 from the positive ESI mass spectrum, showing ion an peak of *m/z* 211 $[M+Na]^+$ and by negative ESIMS showing an ion peak of $[M-H]^-$ at *m/z* 187, which corresponded to the molecular formula $C_{10}H_8N_2O_2$. The ^1H NMR spectrum of **93** exhibited in the aromatic region five 1H signals: a singlet at δ 8.71 (H-2), a doublet at δ 8.28 (H-4), a triplet at δ 7.46 (H-5) and two overlapped proton signals at δ 7.28-7.20 (H-6, 7). The chemical shifts and the signal pattern indicated an indole moiety.

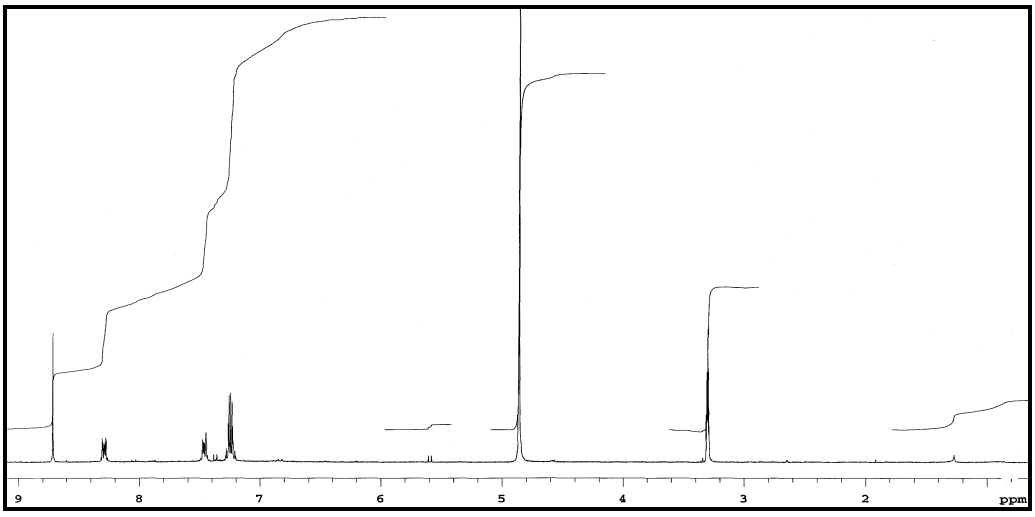


Figure 143: ^1H NMR spectrum (CD_3OD, 300 MHz) of indolyl-3-glyoxylamide (**93**)

The ^{13}C NMR spectrum showed in accordance with the molecular formula 10 carbon signals: eight carbon signals were attributed to an indole moiety and two were carbonyl carbons at δ 183.1 (CO-8) and 168.2 (CO-9).

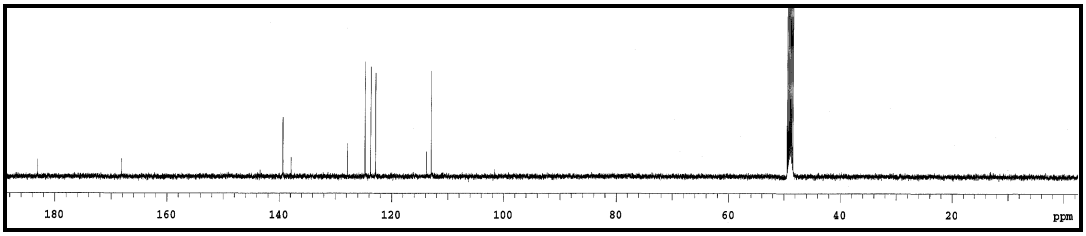
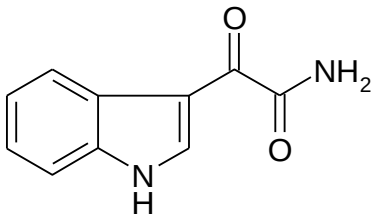


Figure 144: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of indolyl-3-glyoxylamide (**93**)

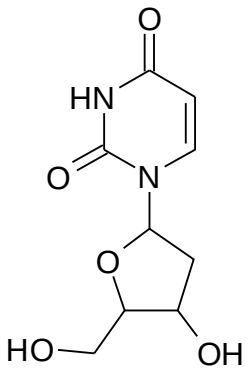
A search in AntiBase[77] and the Chemical Abstract supported by ^1H and ^{13}C NMR data led to indolyl-3-glyoxylamide (**93**), which was isolated previously by Baoquan *et al.*[133] from the marine sponge *Spongosorites* sp. It exhibited cytotoxicity

against a panel of five human solid tumor cell lines and is known as an intermediate in the synthesis of arborescidines [134] and dihydrohamacanthins [135]. It is isolated here for the first time from an *Actinomyces* sp.

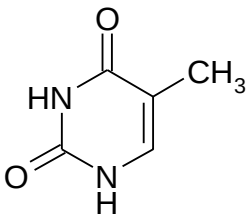


93

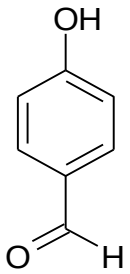
Further trivial compounds isolated from WO 990 were determined as deoxyuridine (**94**), thymine (**95**), 4-hydroxybenzaldehyde (**96**), and indole-3-carbaldehyde (**97**).



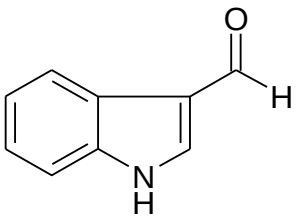
94



95



96



97

4.11 *Bacillus licheniformis*

A culture of *Bacillus licheniformis* was obtained and identified by Jana Tiefenau from University of Braunschweig. The strain was cultivated on LB-medium in a 25 l fermentor to obtain 3.5 g of crude extract. In the chemical screening the crude extract showed on TLC two UV absorbing spots, which gave a black colour with anisaldehyde/sulphuric acid.

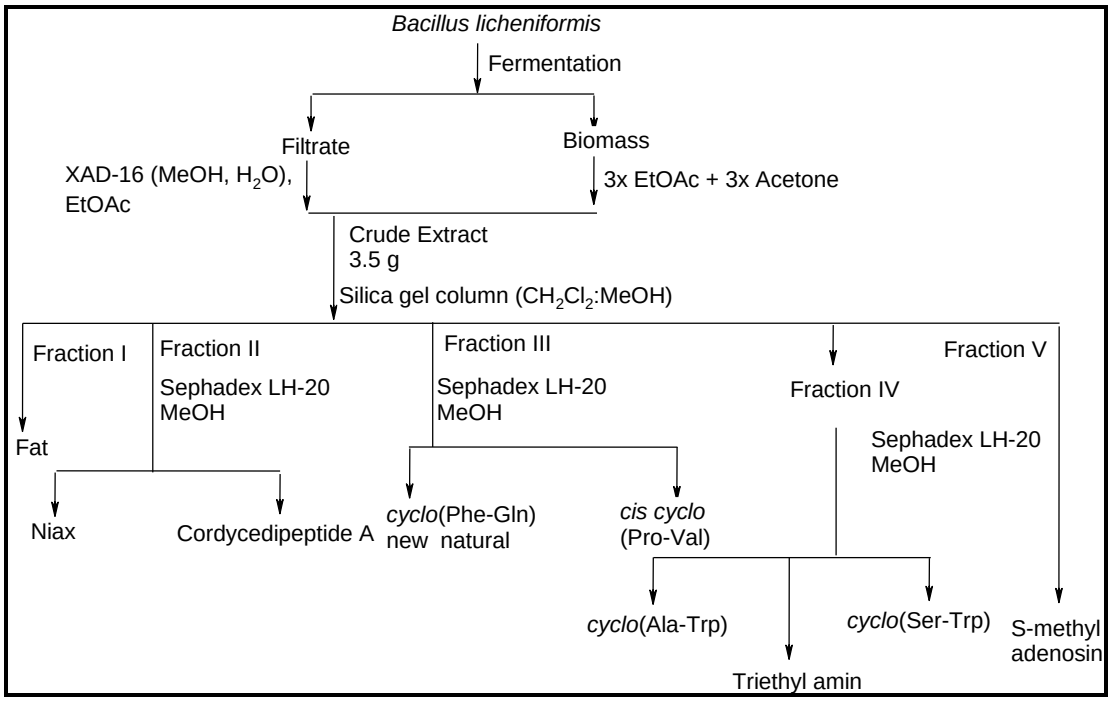


Figure 145: Work-up scheme of *Bacillus licheniformis*

4.11.1 Beauvericin

Beauvericin (**98**) was isolated as a white amorphous solid from the crude extract, exhibiting a blue colouration in the chlorine/tolidine reaction, which is indicative of a peptide structure. The molecular weight of beauvericin (**98**) was established by HPLC MS as 806 Dalton, and the corresponding molecular formula $C_{45}H_{57}N_3O_9Na$ [M+Na] was deduced by HRESIMS. Beauvericin (**98**) is a cyclodepsipeptide toxin produced by several fungi, including *Beauveria bassiana* NRRL 5552.[136] Beauvericin (**98**) and also beauvericin A and B,[137] from *Paecilomyces fumosoroseus,* [138] from *Fusarium roseum acuminatum* or the plant pathogenic fungus *Polyporus sulphureus* [139] showed insecticidal activity[140] and are toxic against mosquito larvae.[137]

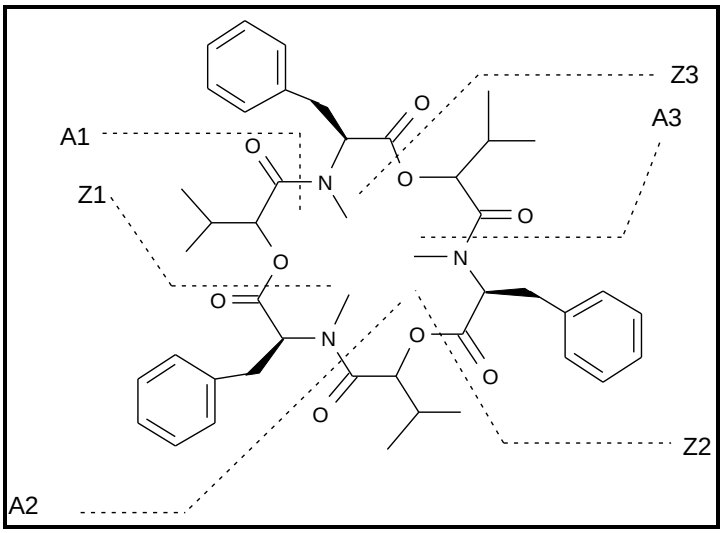


Figure 146: HPLC MS-MS fragments of beauvericin (**98**) [141]

Table 16: HPLC MS-MS fragments of beauvericin (**98**)

m/z observed	Percentage %	Description
243.9	58	$[A1 \text{ to } A2+H-H_2O]^+$
284.0	55	$[A1 \text{ to } A2+Na]^+$
545.1	30	$[A1 \text{ to } A3+Na]^+$
784.3	19	$[M+H]^+$
801.3	22	$[M+NH_4]^+$
806.4	100	$[M+Na]^+$
822.4	10	$[M+K]^+$
384.1	50	$[A1 \text{ to } Z2+Na]^+$

Beauvericin (**98**) is a trimeric lactolide of N-methylphenylalanyl-*α*-hydroxyisovaleryl amide. HPLC MS-MS^3 revealed an ion peak at *m/z* 243, which represented the N-methylphenylalanyl-*α*-hydroxyisovaleryl residue $[A1 \text{ to } A2+H-H_2O]^+$. The ion peak at *m/z* 284 represented the *pseudo*molecular ion of the monomer. Also two other monomers of ester of N-methylphenylalanyl-*α*-hydroxyisovaleryl $[A1 \text{ to } A2+Na]^+$ were observed at *m/z* 545 in HPLC MS-MS^2. HPLC MS-MS afforded molecular ions peak at *m/z* 784 and 801.3, 806.4, 822.4.

4.11.2 *Cyclo*(**Ala,Trp**)

Compound **99** was isolated as a colourless solid from a UV absorbing zone. The ^{1}H NMR spectrum of **99** exhibited five aromatic protons, which were indicative of a 3-substituted indole moiety. The spectrum showed two protons as doublets of dou-

blets at δ 7.58 and 7.30, in addition to two triplets of triplets at δ 7.08, 6.98 and a singlet at δ 7.05. These values are characteristic of an indole moiety. In the aliphatic region signals of two methine protons attached to heteroatoms at δ 4.26, 3.70 were seen. Furthermore, the downfield shift of an ABX system of methylene protons at δ 3.42, 3.17 indicated their connection with an sp^2 carbon or heteroatoms. Also a methyl doublet at δ 0.48 was observed.

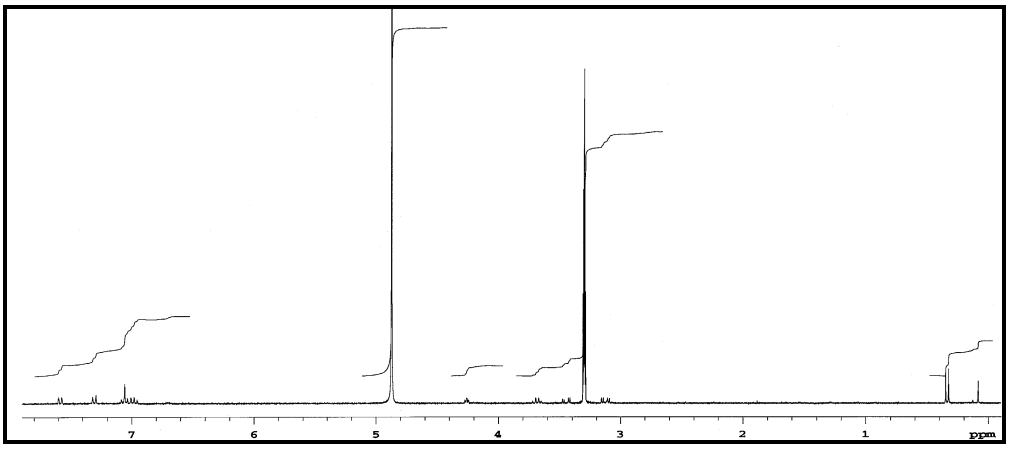


Figure 147: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *cyclo*(Ala,Trp) (**99**)

The ^{13}C NMR spectrum revealed two amide carbonyls of a diketopiperazine at δ 170.4 and 169.3, furthermore 8 carbons of the indole were observed. In addition, two methine carbons attached to nitrogen were displayed at δ 57.5, 51.7, and finally the methylene carbon signal at δ 30.8 and the methyl at δ 20.0 were observed. A search in AntiBase supported by ^{1}H, ^{13}C NMR and MS data led to *cyclo*(Ala,Trp) (**99**) as the result; it was confirmed by comparing with literature data [142]. *Cyclo*(L-Ala,L-Trp) (**99**) was firstly isolated from *Aspergillus chevalieri* sp.[142]

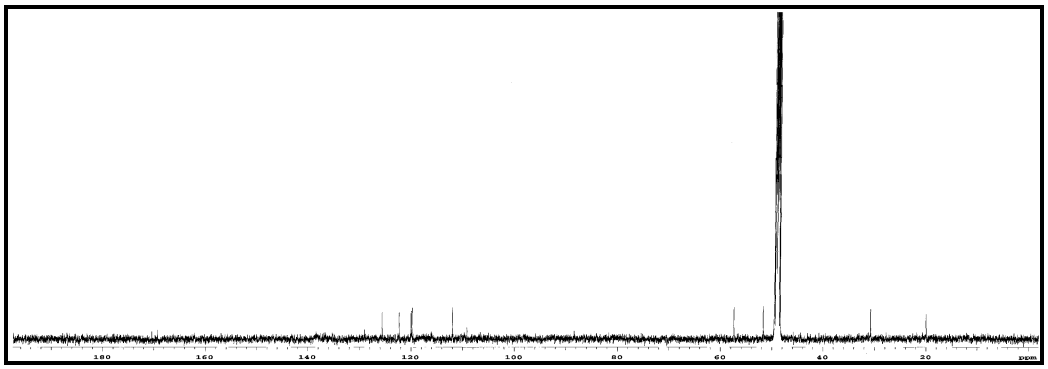
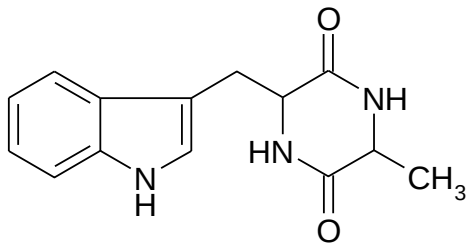


Figure 148: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *cyclo*(Ala,Trp) (**99**)



99

4.11.3 *Cyclo*(**Ser,Trp**)

Cyclo(Ser,Trp) (**100**) was isolated as colourless oily substance from fraction III. The ^{1}H NMR spectrum exhibited in the aromatic region two doublets at δ 7.60, 7.33, and two triplets at δ 7.08, 7.00 respectively, and a singlet at δ 7.10, characteristic of an indole moiety. In the aliphatic region two methine groups attached to heteroatoms were seen at δ 4.21 and 3.81. Furthermore a methylene group appeared as ABX system at δ 3.36 and 2.88, due to its connection with a heteroatom or an sp^2 carbon, in addition to a methylene at δ 3.30. The ESI mass spectrum of compound **100** displayed signals at *m/z* 296 $[M+Na]^+$, 569 $[2M+Na]^+$ in positive mode and *m/z* 272 $[M-H]^-$ and 545 $[2M-H]^-$ in the negative mode. HRESIMS established the molecular formula as $C_{14}H_{15}N_3O_3$.

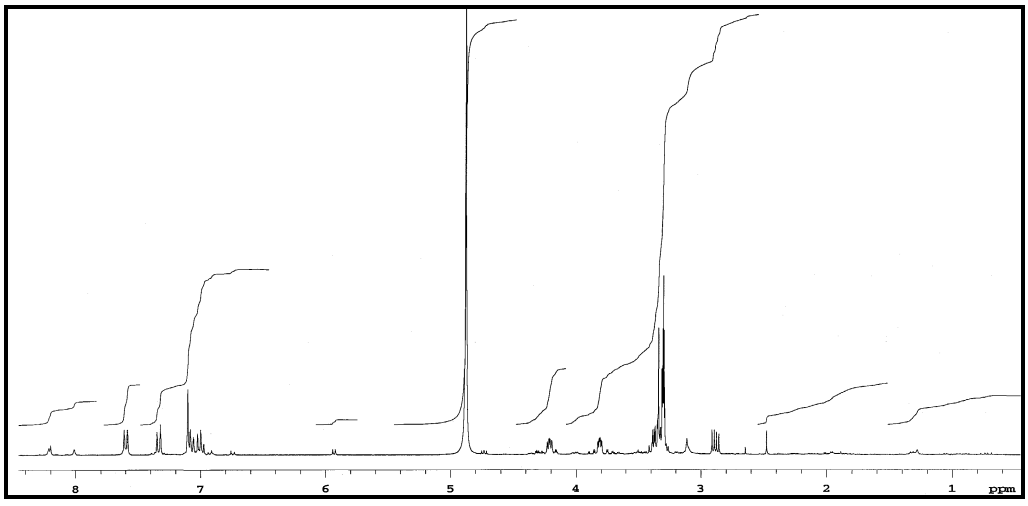


Figure 149: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *cyclo*(Ser,Trp) (**100**)

The ^{13}C NMR and HMQC spectrum exhibited fourteen carbon signals, among them two carbonyl signals at δ 170.0 (C_q-14), 167.9 (C_q-11) for the two carbonyls of a diketopiperazine ring. The remaining carbons in the aromatic region confirmed the

indole moiety. Of the two methylene groups, one at δ 64.7 was connected with a heteroatom, the other one was at δ 32.0. Finally two methine groups at δ 58.8 (CH-12) and 57.3 (CH-9) were present.

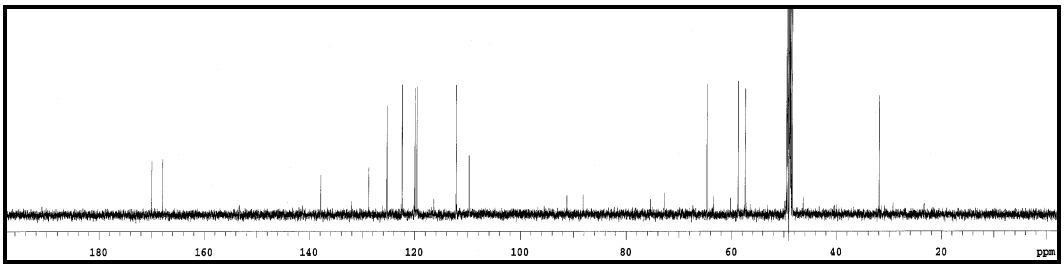


Figure 150: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *cyclo*(Ser,Trp) (**100**)

The ^{1}H,^{1}H COSY spectrum showed the presence of a 1,2-disubstituted benzene ring (**A**); the methine proton at δ 4.21 showed ^3J correlation with a methylene group at δ 3.30 (**B**). In addition, the methine proton at δ 3.81 showed ^3J correlation with methylene protons at δ 3.36 and 2.88 (**C**). The partial structures derived from the ^{1}H NMR and ^{1}H,^{1}H COSY are given below:

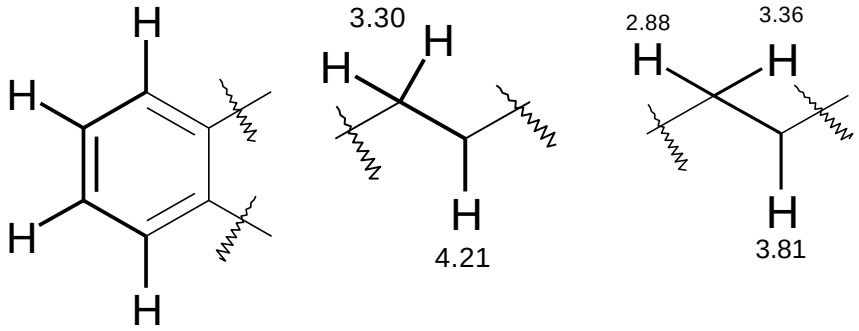


Figure 151: Partial structures derived from ^{1}H,^{1}H COSY data

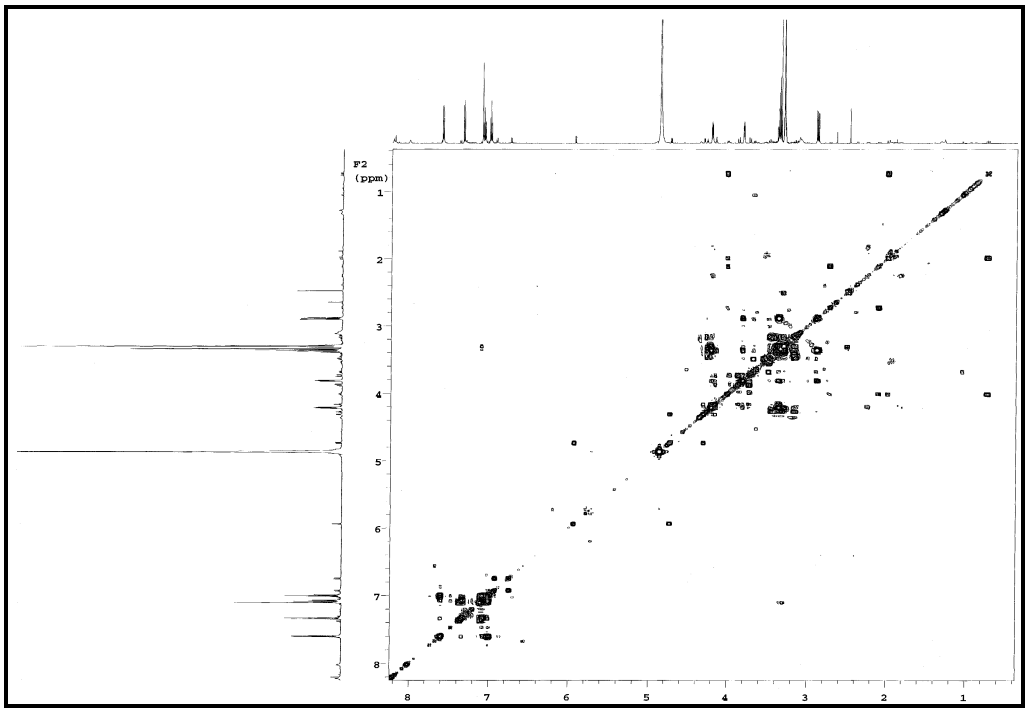


Figure 152: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 600 MHz) of *cyclo*(Ser,Trp) (**100**)

The HMBC spectrum showed a strong correlation of the doublet at δ_H 7.60 with the quaternary carbon at δ_C 137.9 (C_q-7a) and the carbons at δ_C 122.4 (CH-6) and 109.8 (C_q-3). The proton at δ_H 7.00 (CH-5) exhibited ^{3}J correlation to 112.2 (CH-7) and 128.8 (C_q-3a) and the singlet at δ_H 7.10 (CH-2) displayed three bond correlation to C_q-3a (δ_C 128.8) and C_q-7a (δ_C 137.9), confirming the indole moiety. The proton H_2-8 (3.30) showed a three-bond correlation with the carbonyl CO-14 (δ_C 170.0), with CH-2 (δ_C 125.3) and C_q-3 (δ_C 109.8). In addition, the proton H-9 (δ_H 4.21) displayed a three-bond correlation with the carbonyl CO-11 (167.9), while CH-12 (3.81) exhibited ^{3}J correlation to the carbonyl CO-14 (170.0). The methylene protons H_2-15 showed a three-bond correlation with the carbonyl CO-11 (167.9). The detailed interpretation of $^{1}H,^{1}H$ COSY, HMQC, HMBC correlations confirmed the structure of *cyclo*(Ser,Trp) (**100**). A search in AntiBase with these data gave *cyclo*-(Ser,Trp) (**100**) as the result. It was further confirmed by the literature data.[143]

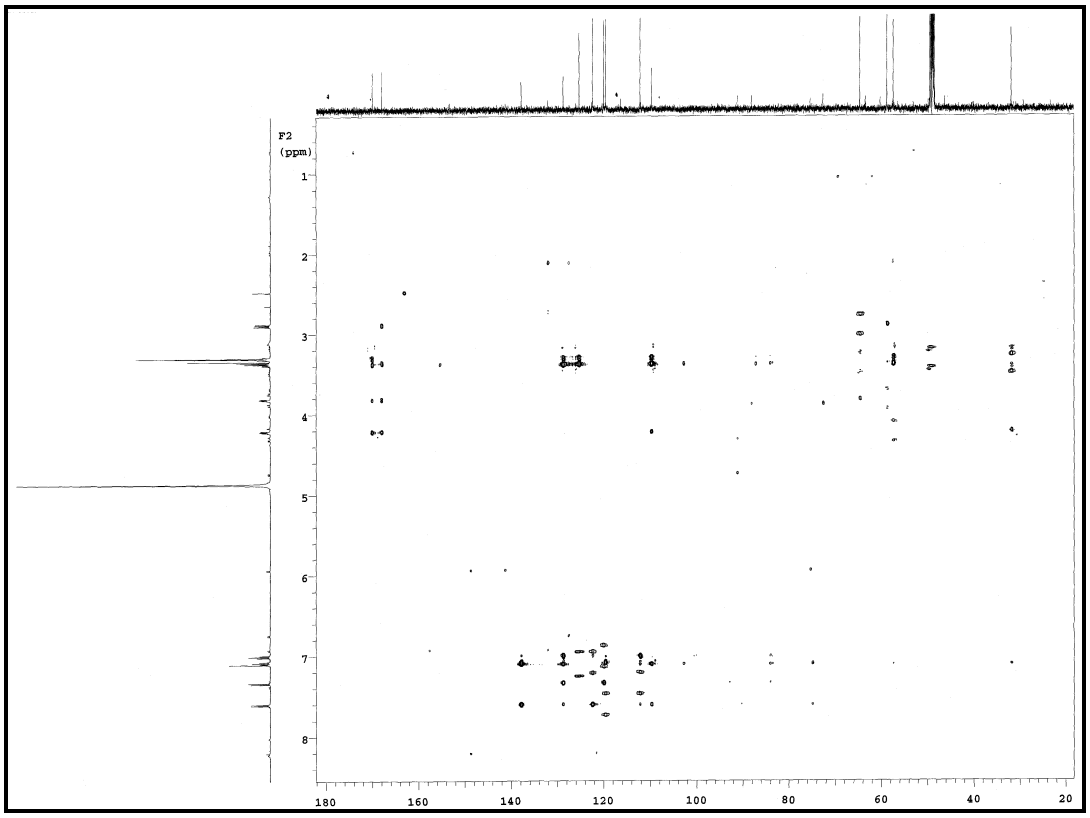
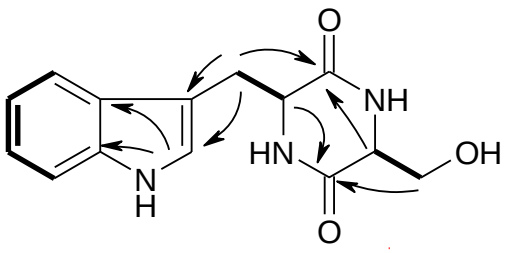


Figure 153: HMBC spectrum of (CD_3OD, 600 MHz) of *cyclo*(Ser,Trp) (**100**).



100

Figure 154: Selected $^1H,^1H$ COSY (▬) and HMBC (→) correlations of *cyclo*-(Ser,Trp) (**100**).

4.11.4 Polypropylenglycol

Polypropylenglycol (**101**) was isolated from fraction III as an oily substance, which was not UV absorbing and gave pink colour on spraying with anisaldehyde/sulphuric acid. The ^1H NMR spectrum revealed in the aliphatic region a methyl doublet at δ 1.12 as well as a methine proton, which overlapped at δ 3.51 with methylene protons. Polypropylenglycol (**101**) is often used as antifoaming agent in fermentor cultures, but frequently it was also isolated as natural product.

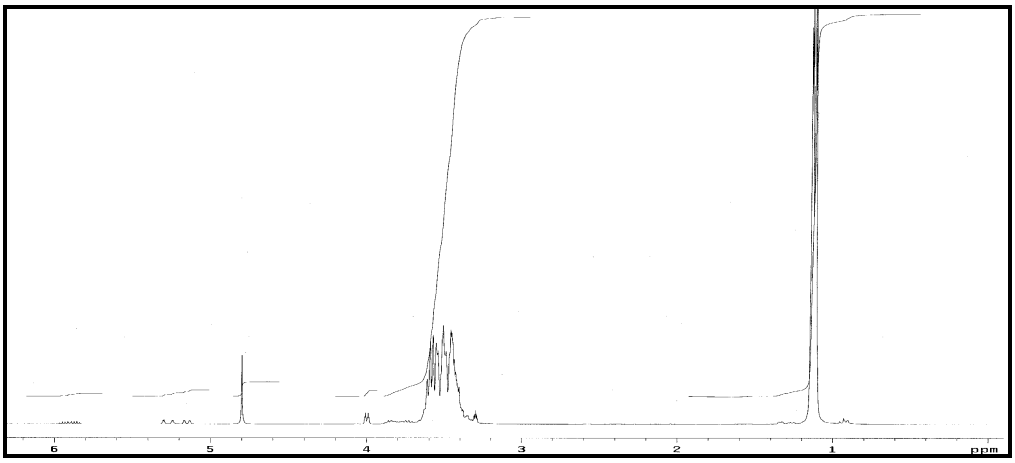
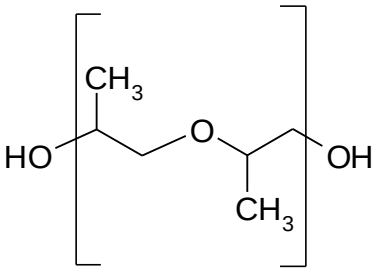


Figure 155: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of polypropylenglycol (**101**)



101

4.11.5 5'-Methylthioadenosine

S-Methyl-thioadenosine (**102**) was isolated as a colourless solid from a UV absorbing band in fraction 6, which gave a brown colour reaction after spraying with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum showed two 1H singlets in the aromatic region at δ 8.31 and 8.19, which suggested an adenine moiety. In the aliphatic region, it displayed a doublet at δ 5.99 (^{3}J = 5.7 Hz) of an anomeric proton, furthermore three oxygenated methines at δ 4.77 (t, ^{3}J = 5.2 Hz), 4.31 (t, ^{3}J = 4.73), and 4.21 (m), and an ABX signal between δ 2.94-2.85 were observed. This resembled the spectrum of adenosine derivatives. In addition, a 3H singlet at δ 2.11 indicative of a methyl group attached to an sp^2 carbon moiety or as a thiomethyl group was present.

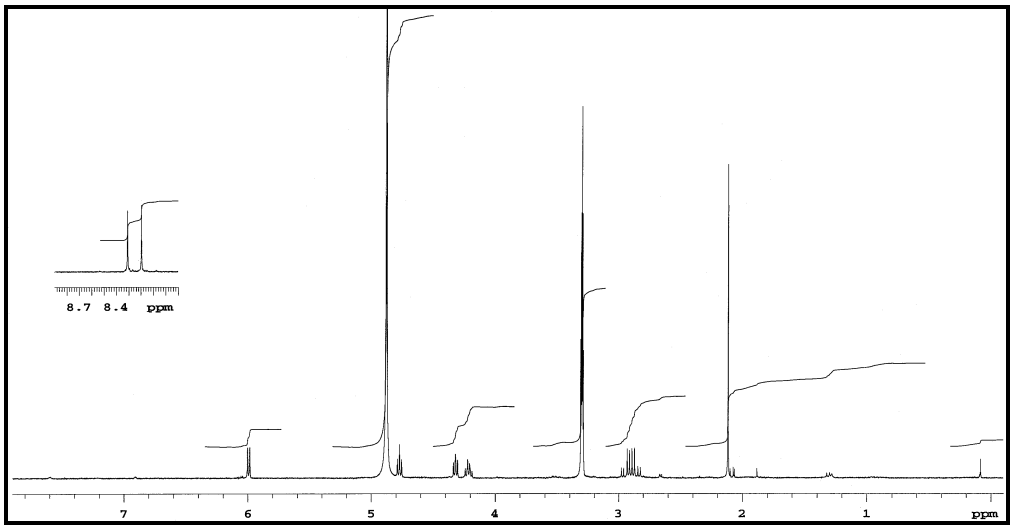


Figure 156: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 5'-methylthioadenosine (**102**)

The ^{13}C NMR spectrum displayed two quaternary carbons attached to heteroatoms at δ 157.2, 150.6, as well as two methine carbons attached to two nitrogen atoms at δ 153.8, 141.2 characteristic of an adenine moiety. An anomeric carbon appeared at δ 90.0. Three oxymethine carbons in the aliphatic region were observed at δ 85.5, 74.9, and 74.0 respectively. In addition, a methylene group at δ 37.5 and a methyl group at δ 16.6 were visible.

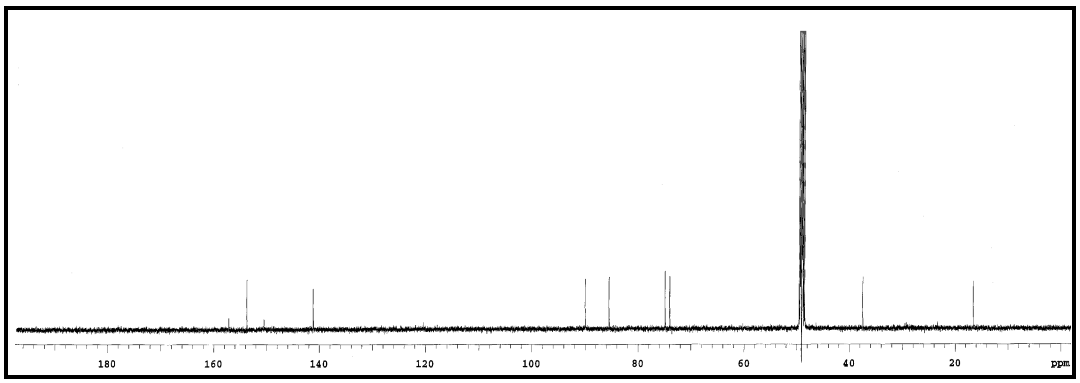
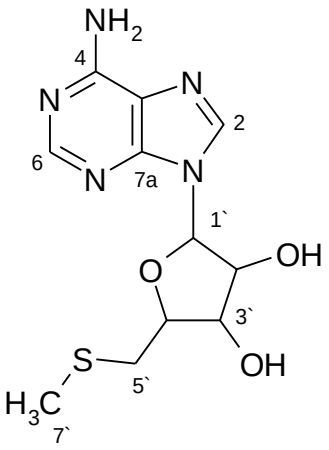


Figure 157: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of 5'-methylthioadenosine (**102**)

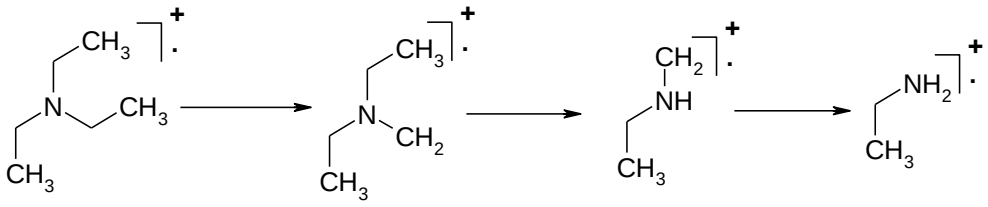


102

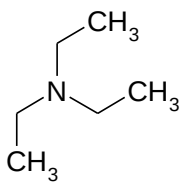
Besides from streptomycetes, 5'-methylthioadenosine (**102**) has been isolated from marine sponges *Aaptos* sp. and *Hymeniacidon aff. heliophila*, and from the nudibranch *Doris aff. verrucosa*.[144]

4.11.6 Triethyl amine

The white crystalline compound **103** showed no UV absorbance and remained colourless with anisaldehyde/sulphuric acid and heating. The aliphatic region of the ^{1}H NMR spectrum revealed only two signals, a quartet at δ 3.22 due to attachment to a heteroatom, and a triplet at δ 1.33. The molecular weight of this compound was determined as 101 Dalton by EIMS. A search in AntiBase with these data gave triethyl amine (**103**) as a result. It was further confirmed by the literature data.[145] The amine was obviously isolated as a salt.



m/z= 101 (30) | *m/z* = 86 base peak | *m/z* = 58 (35) | *m/z* = 36 (20)



103

4.11.7 *Cyclo*(**Phe,Gln**)

Cyclo(Phe,Gln) (**104**) was isolated as a colourless UV absorbing solid from fraction II. The molecular weight of compound **104** was obtained from the ESI mass spectrum, which showed *pseudo*molecular ion peaks at *m/z* 274 $[M–H]^-$ and at *m/z* 298 $[M+Na]^+$, and 573 $[2M+Na]^+$, corresponding to a molecular weight of 275 Dalton. The odd mass number was an indication of an odd number of nitrogen atoms in the compound **104**. HRESIMS established the empirical formula as $C_{14}H_{17}N_3O_3$ and confirmed the presence of three nitrogen atoms. The ^{1}H NMR spectrum revealed in the aromatic region two protons overlapping at δ 7.27 (^3J = 14.9, ^4J = 7.4), in addition to a multiplet with integration of three protons at δ 7.18 indicative of a phenyl group. In the aliphatic region two triplets at δ 4.16 and δ 3.64 of methine protons attached to nitrogen were present. Furthermore two methylene groups were observed at δ 3.10, 2.89 and 1.35, 1.02 as two ABX systems, with the first one connected with an sp^2 carbon or a heteroatom. Finally a methylene triplet was displayed at δ 1.68.

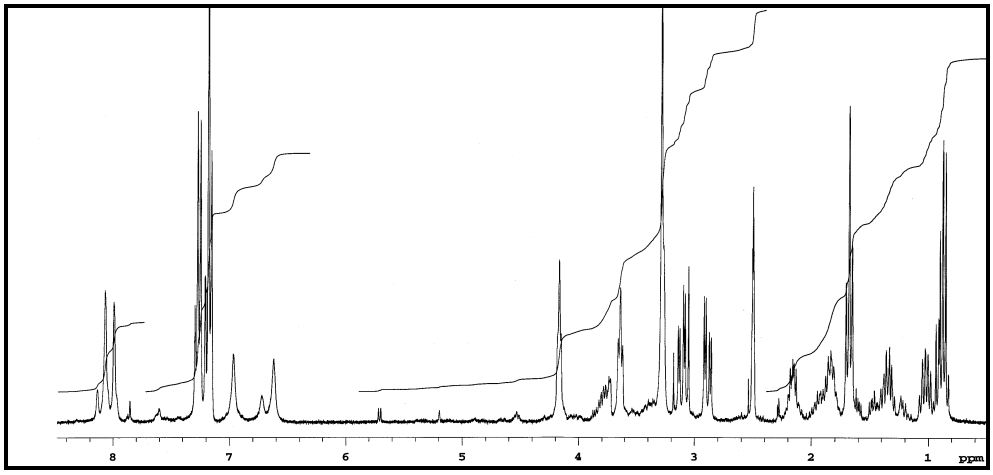


Figure 158: ^{1}H NMR spectrum (DMSO-d_6, 500 MHz) of *cyclo*(Phe,Gln) (**104**)

The ^{13}C NMR spectrum revealed three carbonyl signals at δ 173.2 (Cq-3''), 166.7 (Cq-2), 166.2 (Cq-5), which were attributed to acid derivatives. Six further carbon signals were observed at δ 136.0 (C_q-1'), 130.0 (CH-2', CH-6'), 127.8 (CH-3', CH-5'), 126.4 (CH-4'), which suggested a benzene ring. In addition, two methine carbons attached to a heteroatom at δ 55.2, 53.3 were observed. Finally three methylene carbons at δ 38.2 (CH_2-7), 30.2 (CH_2-2'') and 29.3 (CH_2-1'') were present.

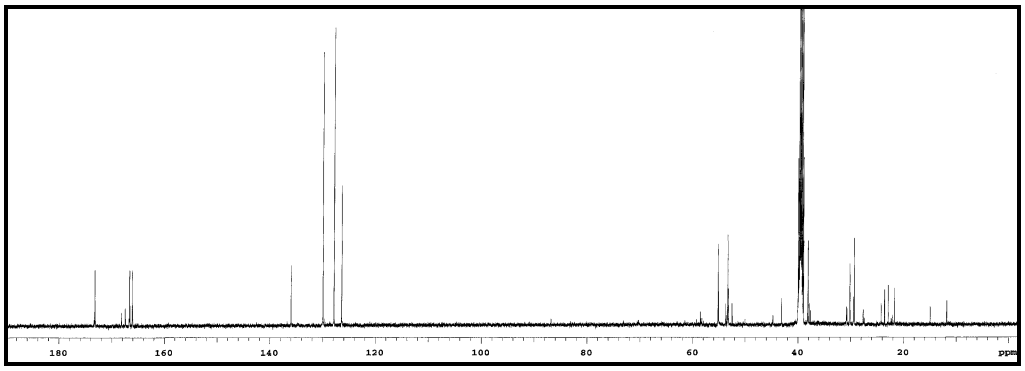


Figure 159: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of *cyclo*(Phe,Gln) (**104**)

The ^{1}H,^{1}H COSY spectrum showed the presence of a mono-substituted benzene ring (fragment A), as well as a methine proton at δ 3.64 which exhibited ^{3}J correlation to the methylene protons at δ 3.10 and 2.89 (fragment B). In addition, a methine proton at δ 4.16 showed ^{3}J correlation with a methylene group at δ 1.35 (H-1"a) and 1.02 (H-1"b) (fragment C). The partial structures derived from the ^{1}H NMR and ^{1}H,^{1}H COSY are given below:

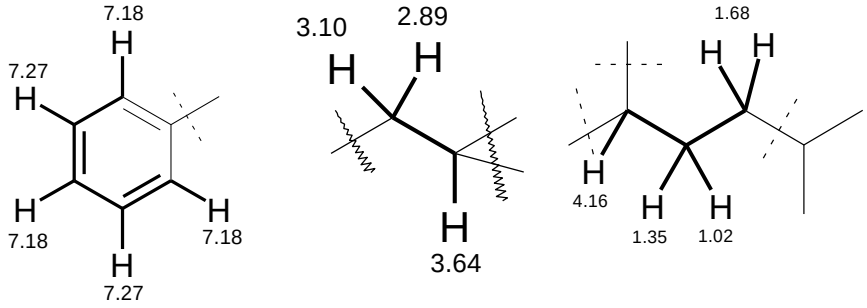


Figure 160: Selected of ^{1}H,^{1}H COSY correlations (▬) for *cyclo*(Phe,Gln) (**104**)

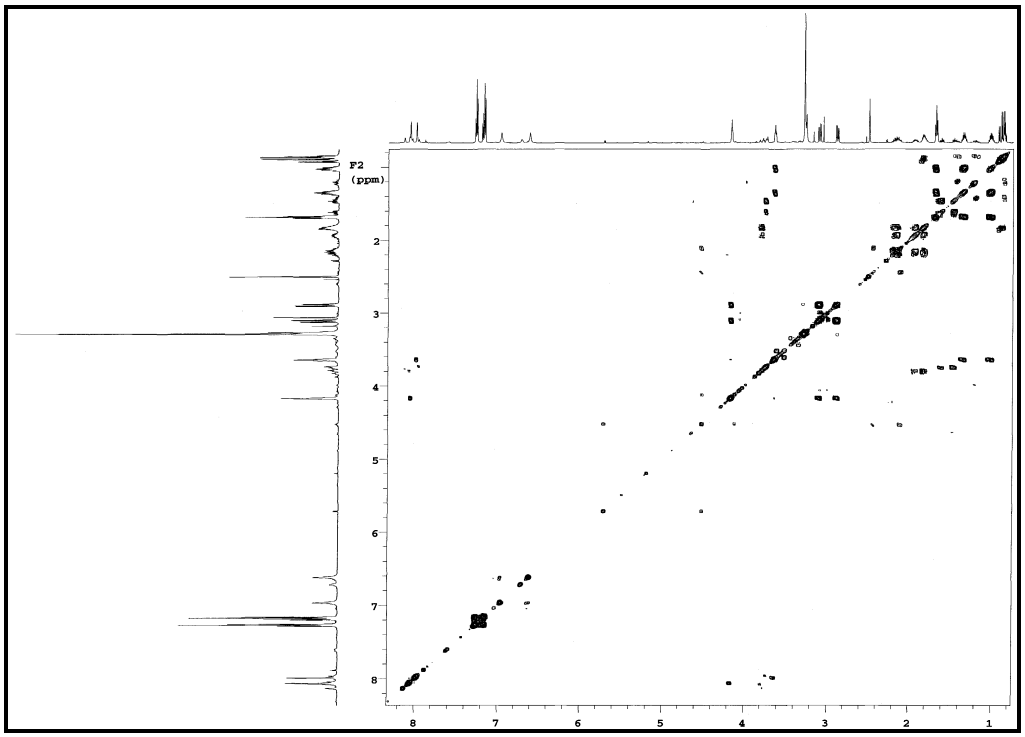
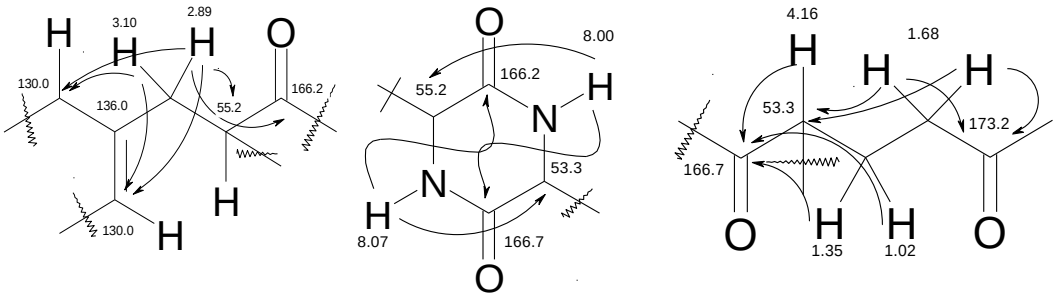


Figure 161: ^{1}H,^{1}H COSY spectrum (DMSO-d_6, 500 MHz) of *cyclo*(Phe,Gln) (**104**)



A **B** **C**

Figure 162: Substructures and selected HMBC (→) correlations of *cyclo*(Phe,Gln) (**104**)

The HMBC spectrum of **104** exhibited strong correlations from the methylene protons at δ 3.10 and 2.89 to two sp^2 methine carbons at δ 130.0 (intensity of two carbon) and to a quaternary carbon at δ 136.0 in benzene ring, as well as to a methine carbon at δ 56.2 and to carbonyl amide at δ 166.2 (Fragment **A**). The acidic proton at δ 8.07 displayed HMBC correlations to the carboxamide at δ 166.2 and the methine carbon at δ 53.3 in (Fragment **B**) while the acidic proton at δ 8.00 showed strong ^{3}J correlation to the carboxamide at δ 166.7 and the methine carbon at δ 55.2 to confirm a piperazinedione moiety attached to fragment **A**. The HMBC spectrum also showed

three-bond correlations of the methylene protons at δ 1.35 and 1.02 to a carbonyl amide at δ 166.7, which showed also strong correlation from the methine at δ 4.16. The methylene triplet at δ 1.68 exhibited a ^3J cross linkage to the carbonyl at δ 173.2 (Fragment **C**).

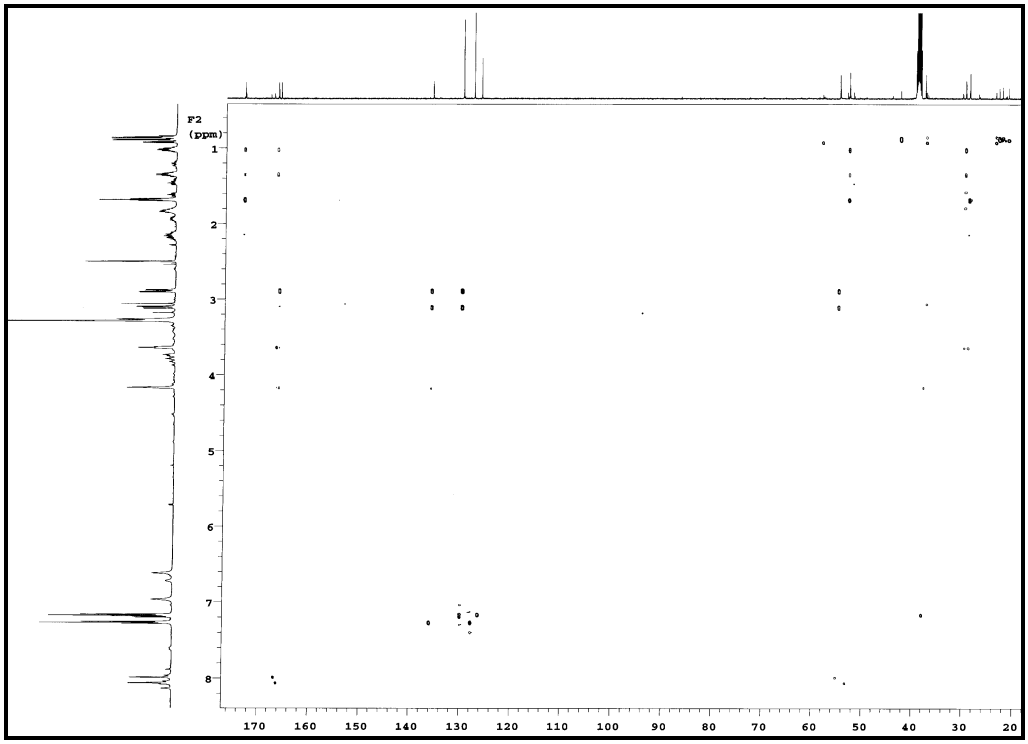
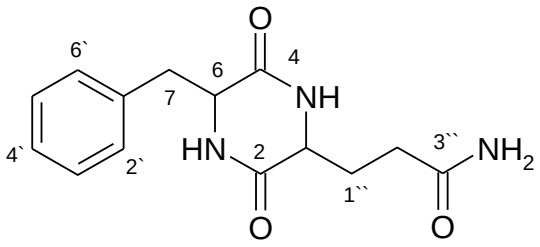


Figure 163: HMBC spectrum (DMSO-d_6, 500 MHz) of *cyclo*(Phe,Gln) (**104**)



104

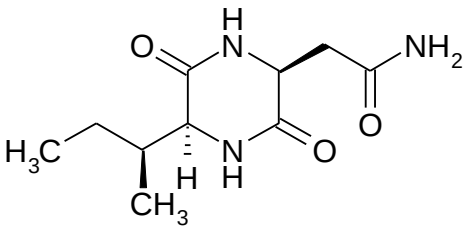
A search in AntiBase and the Chemical Abstract with these data suggested *cyclo*-(Phe,Gln) (**104**), which is a new natural compound. It was synthesized according to Wang *et al.*[146] Diketopiperazins are the smallest cyclic peptides, and are commonly biosynthesised from amino acids by different microorganisms, including bacteria, and are considered to be secondary metabolites.[147] Some of the most important biological activities of diketopiperazine are related to the inhibition of plasminogen activator inhibitor-1 (PAI-1)[148,149] and the alteration of cardiovascular and blood-

clotting functions.[150] Diketopiperazines containing glutamine or glutamic acid were so far not found in nature.

4.11.8 Cordycedipeptide A

The colourless solid **105** was isolated as UV active compound, which changed to blue with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of **105** exhibited in the aromatic region four exchangeable protons, a broad singlet at δ 7.98, a singlet at δ 7.68, and further broad singlets at δ 7.40 and 6.91 for an NH_2 group. In the aliphatic region two methyl groups were visible: one as a triplet at δ 0.85 and the other one as doublet at δ 0.93. Furthermore two methylene signals were observed at δ 1.21, 1.42 and 2.69, 2.31 as part of two ABX systems. The downfield shift for the second one indicated that it was in connection with an sp^2 carbon or a heteroatom. In addition, three methine groups were present, giving a multiplet at δ 1.85 and two multiplets at δ 4.19 and 3.77.

The ^{13}C NMR spectrum of **105** exhibited three quaternary carbon signals at δ 171.8, 167.4, 166.5 for amide, ester or acid carbonyl groups. In the aliphatic region two methine carbons attached to nitrogen atoms appeared at δ 58.3 and 50.8, and another methine carbon at δ 37.5 was found. Furthermore, two methylene groups at δ 38.5 (connected to sp^2 carbon) and δ 24.1, as well as methyl groups at δ 15.0, 11.8 were observed. The ESI mass spectrum of this compound displayed signals at *m/z* 226 $[M-H]^-$, 453 $[2M-H]^-$ and 250 $[M+Na]^+$. According to these spectroscopic data and a search in AntiBase and by comparing with literature data this compound was determined as cordycedipeptide A (**105**).[151]



105

4.12 *Bacillus subtilis* MZ 6

The strain *Bacillus subtilis* MZ 6 was selected based on the chemical and biological screening. The TLC analysis exhibited different coloured spots with anisaldehyde and Ehrlich's reagent, and with the chlorine/anisidine reaction. The crude extract showed good biological activities against different microorganisms as mentioned in Figure 256.

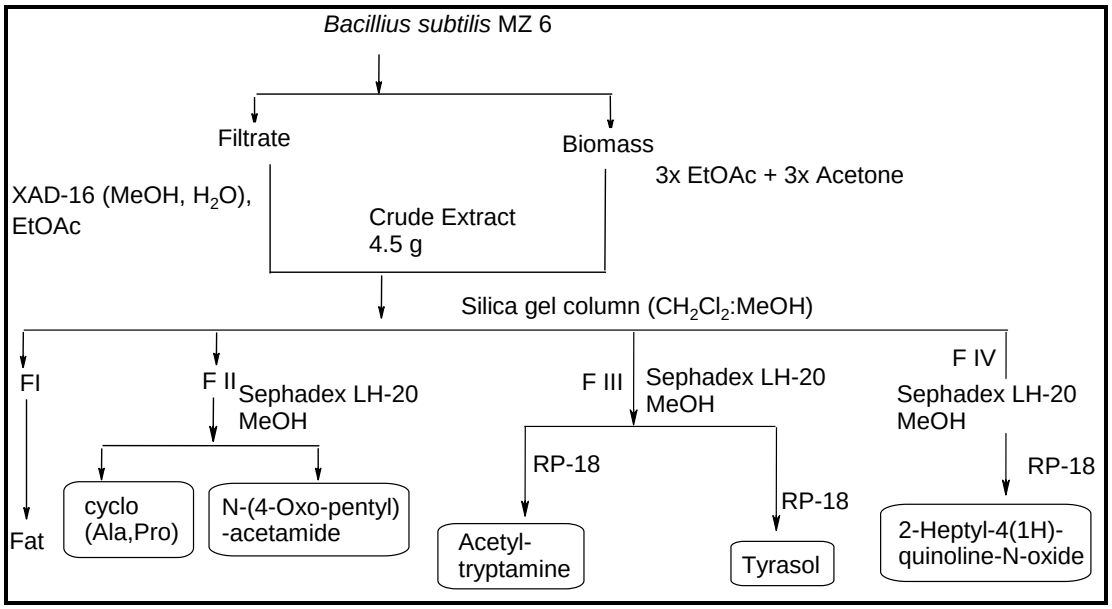


Figure 164: Work-up for scheme for *Bacillus subtilis* MZ 6

4.12.1 *Cyclo*(**Ala,Pro**)

Cyclo(Ala,Pro) (**106**) was isolated as a colourless solid from fraction II. The ^{1}H NMR spectrum of **106** exhibited in the aliphatic region two overlapping methine signals at δ 4.22, which indicated the connection with heteroatoms, as well as one methylene group at δ 3.50 attached to a nitrogen atom. Furthermore, two methylene multiplets were observed at δ 2.28 and 1.99, representing 1H and 3H, respectively, and a methyl doublet at δ 1.37 was present.

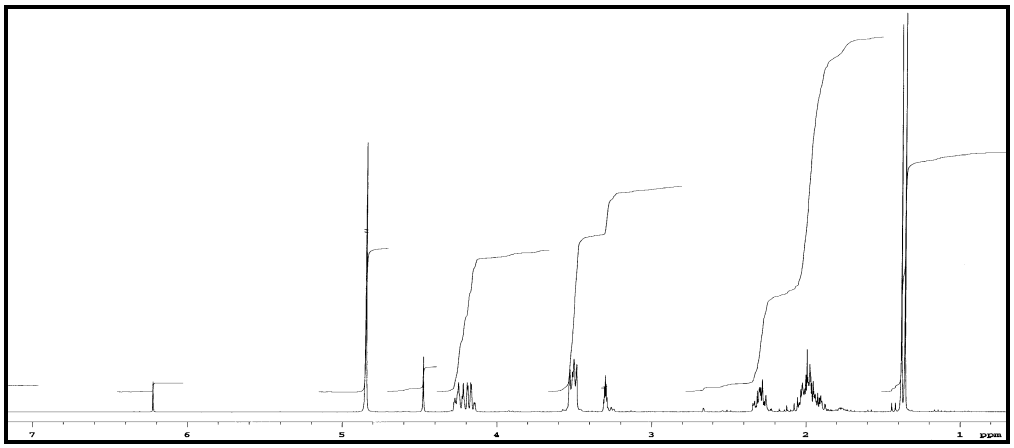


Figure 165: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *cyclo*(Ala,Pro) (**106**)

The ^{13}C NMR spectrum of **106** showed 8 carbon signals, among them two peaks at δ 172.6, 169.0 for two carbonyls, as well as two methine carbons attached to nitrogen at δ 60.5 and 52.1. Furthermore three methylene groups at δ 46.4, 29.2 and 23.6 and a methyl carbon signal at δ 15.7 were observed. A search in AntiBase with these data gave *cyclo*(Ala,Pro) (**106**) as a result. It was further confirmed by the literature data and comparing with authentic spectra.

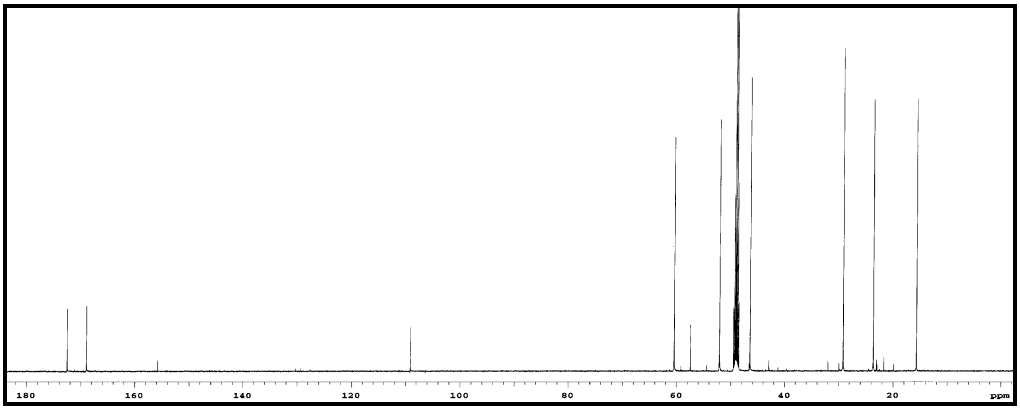
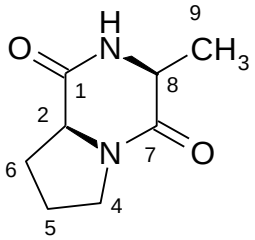


Figure 166: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *cyclo*(Ala,Pro) (**106**)



106

4.12.2 N-(4-Oxo-pentyl)-acetamide

Compound **107** was isolated as a colourless solid with no UV absorbance from fraction FII. The molecular formula was established as $C_7H_{14}NO_2$ according to the HRESIMS. The ^{1}H NMR spectrum showed in the upfield region three methylene groups: a quartet at δ 2.98, a triplet at δ 2.41 and a multiplet centred at δ 1.56; in addition two methyl singlets appeared at δ 2.06 and 1.77, respectively.

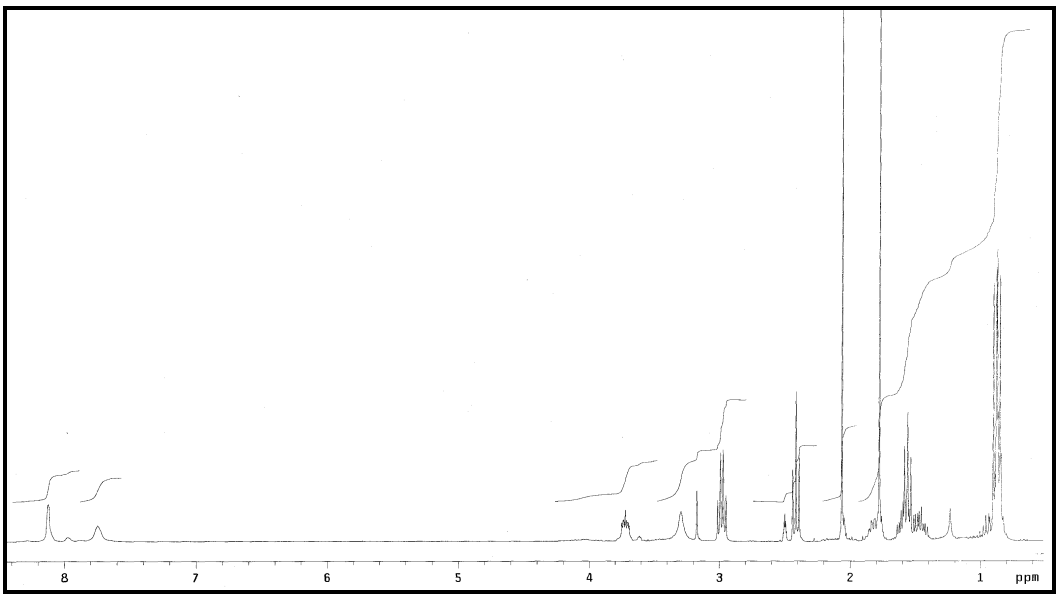


Figure 167: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of N-(4-oxo-pentyl)-acetamide (**107**)

The ^{13}C NMR spectrum of **107** revealed seven carbon atoms including a ketone carbonyl at δ 207.9, a carbonyl of an ester, acid or amid at δ 170.0, along with three methylene carbons at δ 40.0, 37.8 and 29.3, and two methyl carbons at 23.3 and 22.5. These assignments were confirmed by the HSQC spectrum.

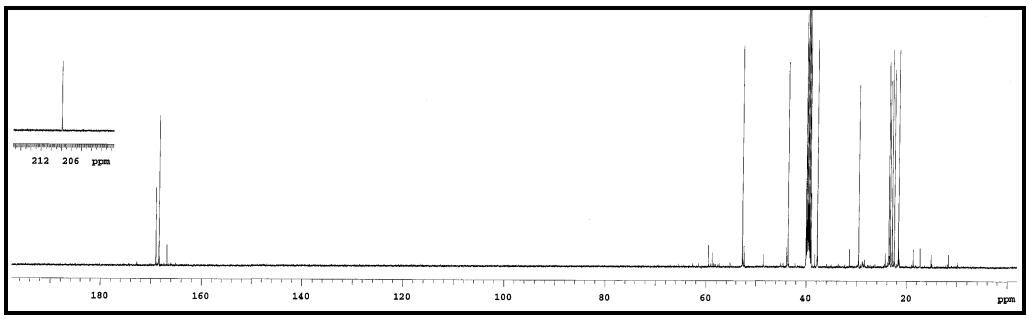


Figure 168: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of N-(4-oxo-pentyl)-acetamide (**107**)

A search in AntiBase supported by ^{1}H, ^{13}C NMR spectroscopic data afforded no hits. To confirm that compound **107** was a new natural product, 2D NMR measurements were performed. The ethylene protons at δ 1.65 had a strong COSY coupling with methylene groups at δ 2.41 and 2.98, which gave a propandiyl fragment (-CH_2-CH_2-CH_2-).

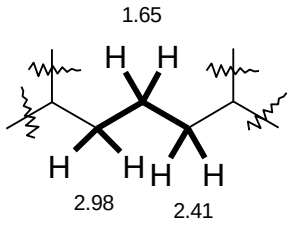


Figure 169: Selected ^{1}H,^{1}H COSY (▬) of N-(4-oxo-pentyl)-acetamide (**107**)

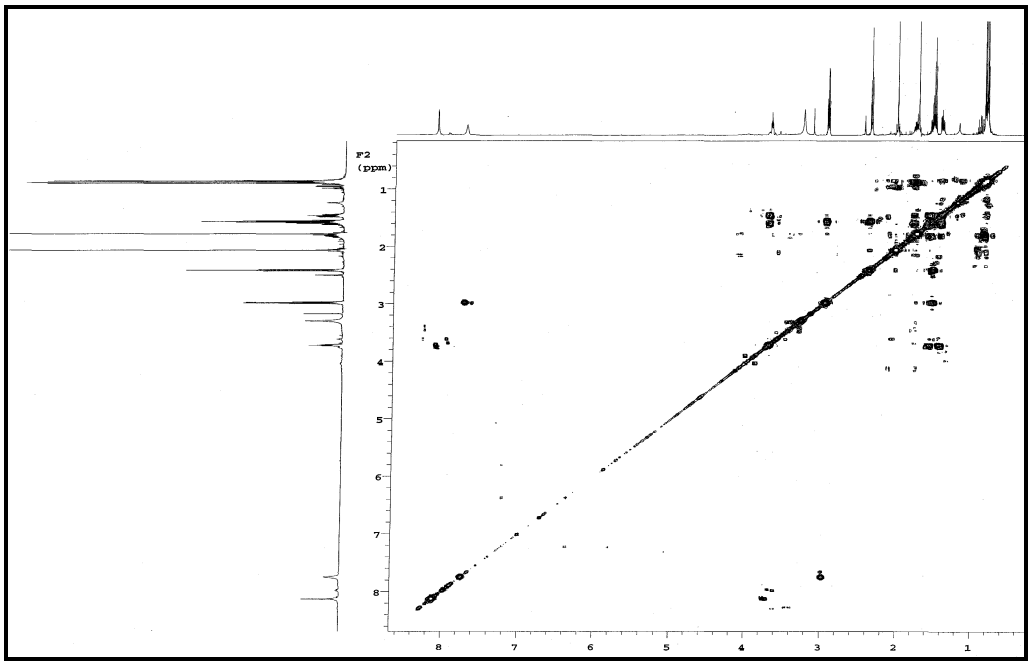
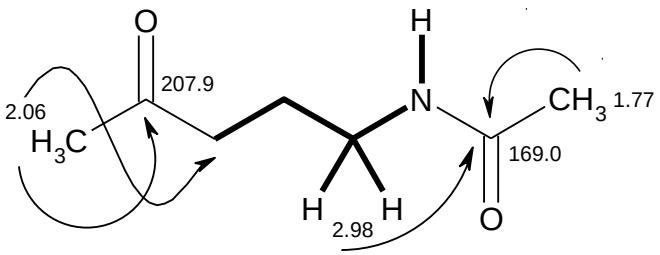


Figure 170: ^{1}H,^{1}H COSY spectrum (DMSO-d_6, 500 MHz) of N-(4-oxo-pentyl)-acetamide (**107**)

The HMBC spectrum exhibited ^{2}J correlation from the methyl singlet at δ 2.06 to a ketone carbonyl at δ 207.9 and ^{3}J correlation to methylene protons at δ 2.41, which themselves correlated to the ketone carbonyl and the methylene protons at δ 1.65. On the other hand methyl protons at δ 1.77 and the methylene protons at δ 2.98 showed HMBC correlations with the amide carbonyl at δ 170.0. A contamination was identified as leucine by shift values and 2D correlations, but could not be separated.



107

Figure 171: Selected ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of N-(4-oxo-pentyl)-acetamide (**107**)

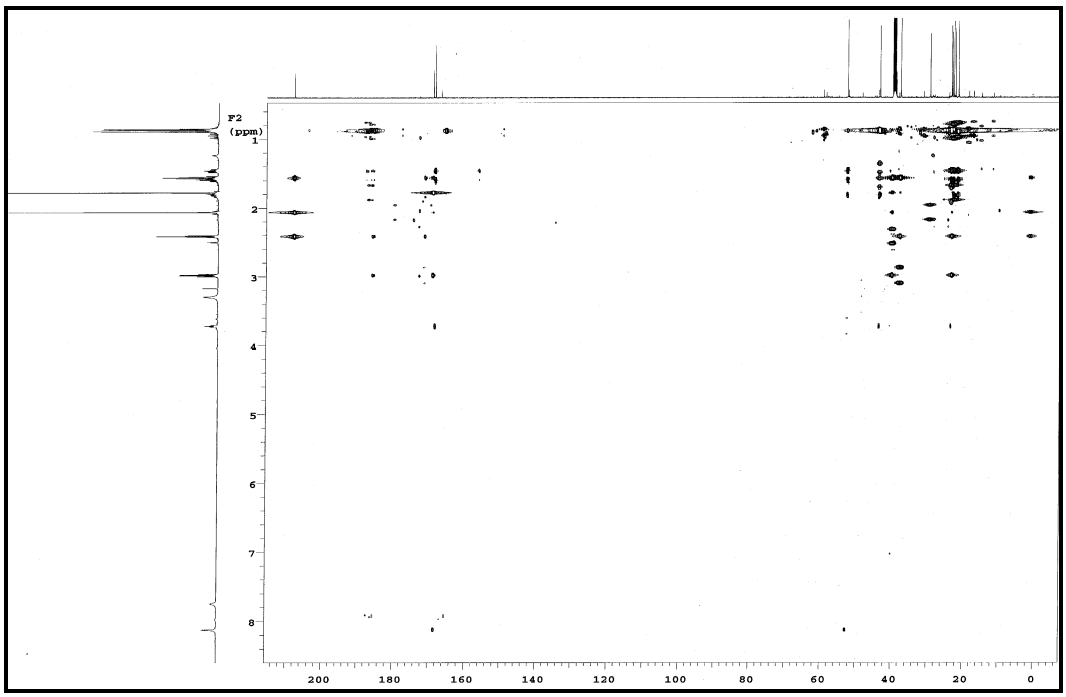
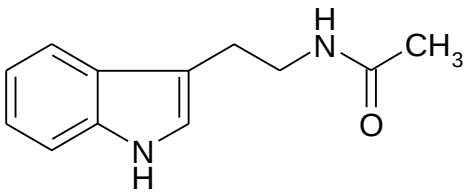


Figure 172: HMBC spectrum (DMSO-d_6, 500 MHz) of N-(4-oxo-pentyl)-acetamide (**107**)

4.12.3 Nβ-Acetyl tryptamine

The ^{1}H NMR spectrum **108** exhibited in the aromatic region the pattern of a 3-substituted indole nucleus. In the aliphatic region two methylene groups gave triplets at δ 3.41 (^{3}J = 11.4, 3.9 Hz) and 2.89 (^{3}J = 14.6, ^{4}J = 7.1 Hz); a methyl singlet at δ 1.85 pointed to the attachment to an sp^2 carbon. A search in AntiBase with these data gave N^{ß}-acetyl-tryptamine (**108**) as a result. It was further confirmed by the literature data [152, 153] and by comparing with authentic spectra.



108

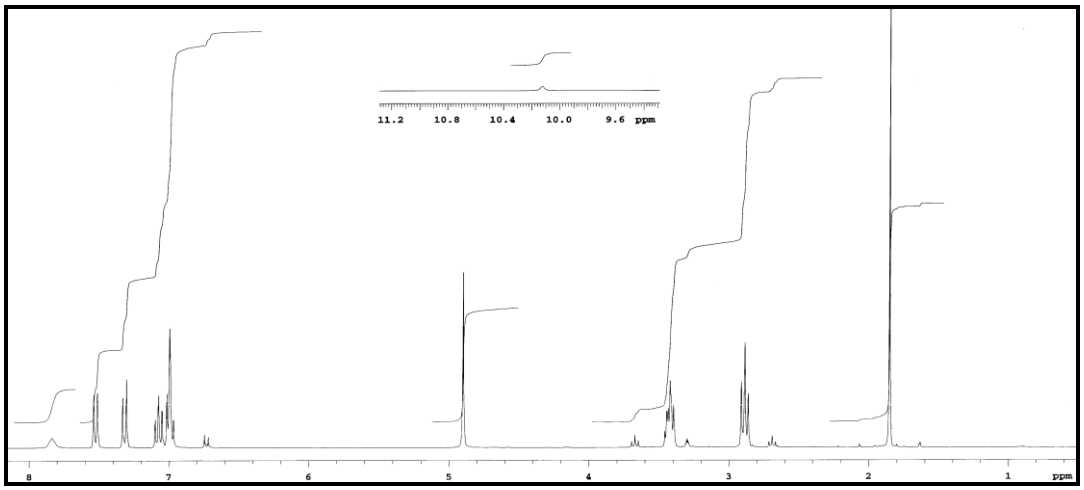


Figure 173: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of N^{β}-acetyltryptamine (**108**)

Tryptamine and its derivatives are widely distributed in bacteria, fungi, animals, and plants:[154,155,156] Tryptamine can be found in edible fruits such as tomato, plums, and eggplant fruits, and is also present in small quantities in oranges.[157]

4.12.4 Tyrosol

The ^{1}H NMR spectrum of **109** exhibited in the aromatic region 2H doublets at δ 7.01 and 6.69, which is characteristic of a 1,4-disubstituted benzene ring. In the aliphatic region, two methylene groups were present as triplets at δ 3.67 and 2.70. A search in AntiBase with these data gave tyrosol (**109).** It was further confirmed by the literature data and comparing with authentic spectra.

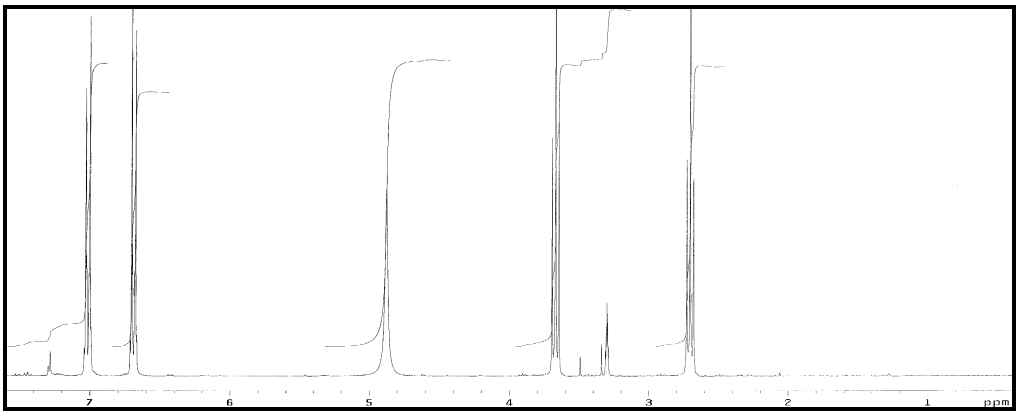
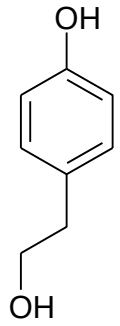


Figure 174: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of tyrosol (**109**)



109

4.12.5 2-Heptyl-4 (1H)-quinolinone-N-oxide

Compound **110** was isolated as colourless solid, which exhibited UV absorbance at 254 nm and turned to yellow with anisaldehyde and sulphuric acid and heating. The ^{1}H NMR spectrum of **110** exhibited in the offset a broad signal of an exchangeable proton at δ 11.67, in addition to four aromatic protons indicative of a 1,2-disubstited benzene ring. Two of them appeared as doublets of doublets at δ 8.25 (J = 0.9, J = 8.0 Hz) and at δ 8.08 (J = 0.9, J = 8.0 Hz) and two protons gave doublets of triplets at δ 7.58 (J = 1.3, J = 8.4 Hz) and 7.36 (J = 0.7, J = 8.0 Hz). An olefinic 1H singlet was observed at δ 6.37. In the aliphatic region, two methylene groups appeared at δ 2.77, 1.75 and four methylene groups of a chain overlapped at δ 1.70-1.20; a methyl triplet was present at δ 0.86.

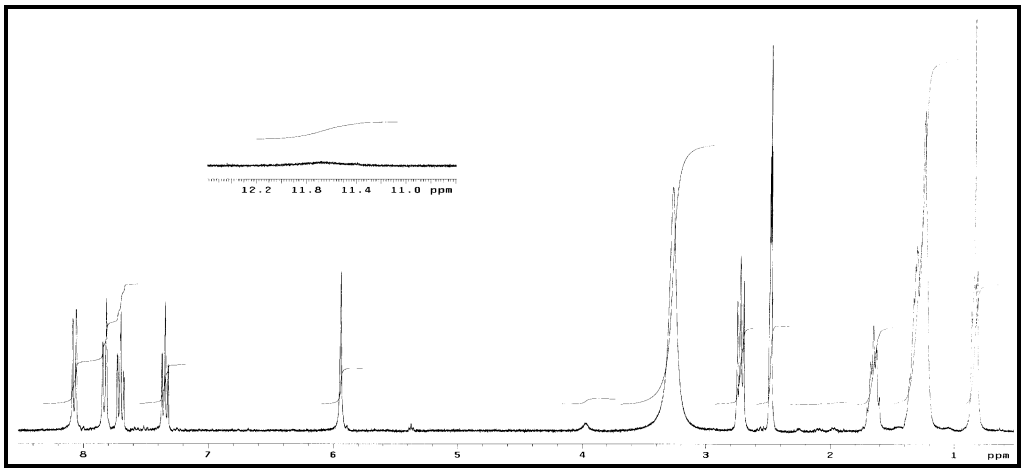


Figure 175: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of KF8940 (**110**)

Based on the ^{13}C NMR and HMQC Spectrum of **110,** 16 carbon signals were visible, among them the carbonyl of an acid, amide or ester at δ 174.0; a quaternary carbon at δ 156.4 attached to a heteroatom and two quaternary carbons were displayed in the sp^2 region at δ 142.0 and 125.4. Additionally, five methine sp^2 carbons as well as six overlapped methylene carbons and a methyl signal at δ 14.4 were observed.

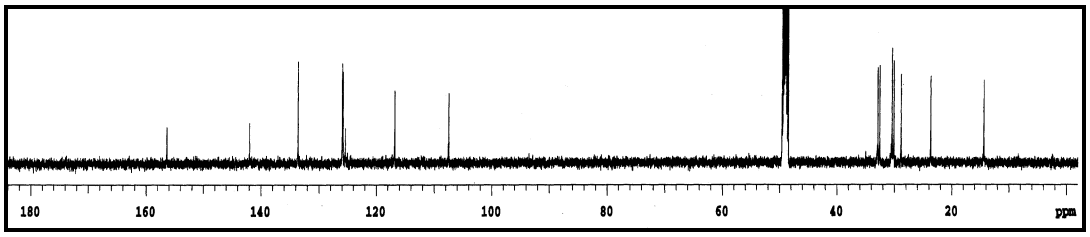


Figure 176: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of KF8940 (**110**)

The ^{1}H,^{1}H COSY correlations showed a 1,2-disubstituted benzene ring, where a proton at δ 8.25 (d, H-5) showed ^{3}J correlation with a signal at δ 7.58 (H-6), which appeared as triplet. The latter showed a strong coupling (^{3}J) with a proton at δ 7.36 (H-7), while this proton exhibited strong correlation with a signal at δ 8.08 (fragment **A**). The methylene group at δ 2.77 (H_2-1') exhibited ^{3}J coupling with δ 1.77 (H_2-2'), and the latter showed a three-bond correlation to the methylene at δ 1.29.

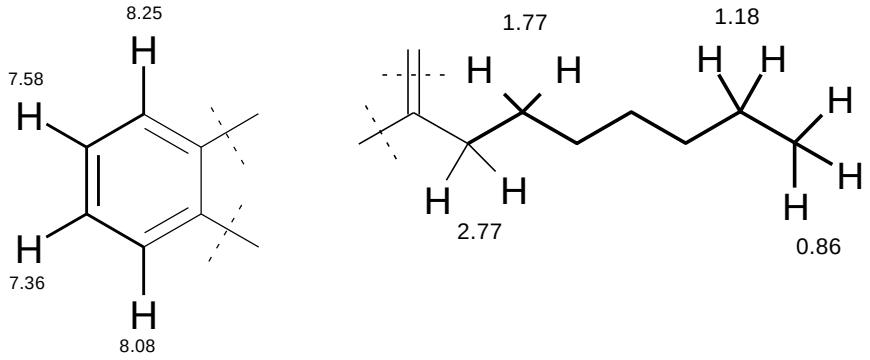


Figure 177: ^{1}H,^{1}H COSY fragments (▬) of KF8940 (**110**)

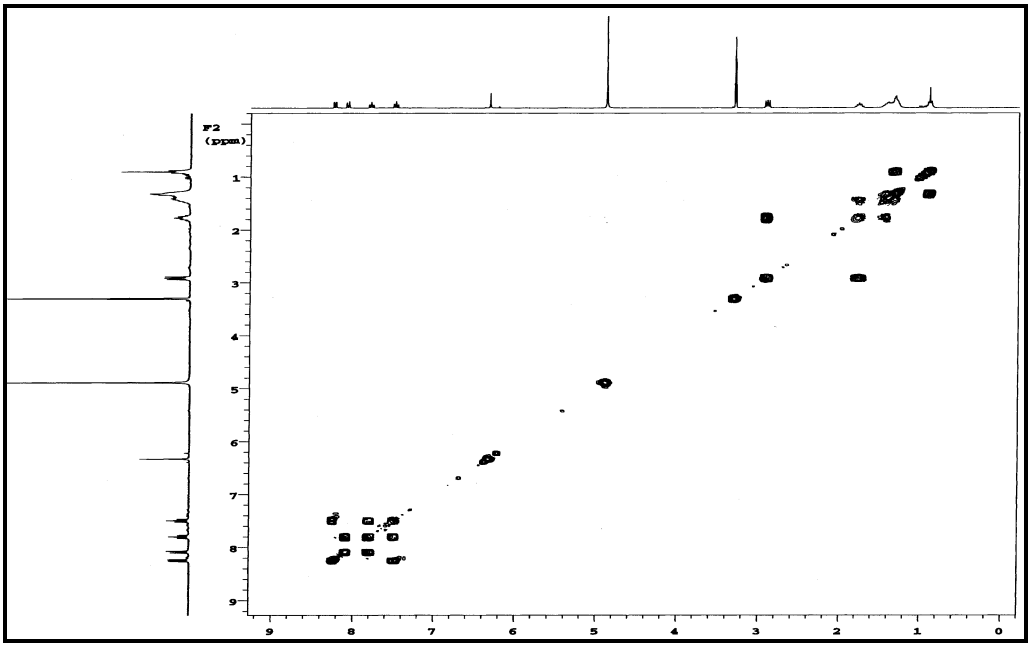


Figure 178: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of KF8940 (**110**)

Based on the HMBC spectrum, a doublet at δ 8.25 displayed correlations with the carbonyl at δ 174.0 and quaternary carbons at δ 125.4 (C_q-4a), δ 142.0 (C_q-8a), δ 133.6 (CH-7). The triplet at δ 7.38 exhibited strong correlation to the quaternary carbon at δ 142.0, to confirm the 1,2-disubstituted benzene ring. The singlet at δ 6.37 showed ^{2}J coupling with the carbonyl at δ 174.0 and the quaternary carbon at δ 156.4 and showed ^{3}J cross linkage to quaternary carbon at δ 125.4 and to the methylene carbon at δ 32.6 (CH_2-1'), which suggested the side chain linkage to the quaternary carbon at δ 156.4. The HMBC spectrum also showed a correlation of the terminal methyl protons at δ 0,86 with two methylene carbons at δ 14.4 and δ 23.7 respectively.

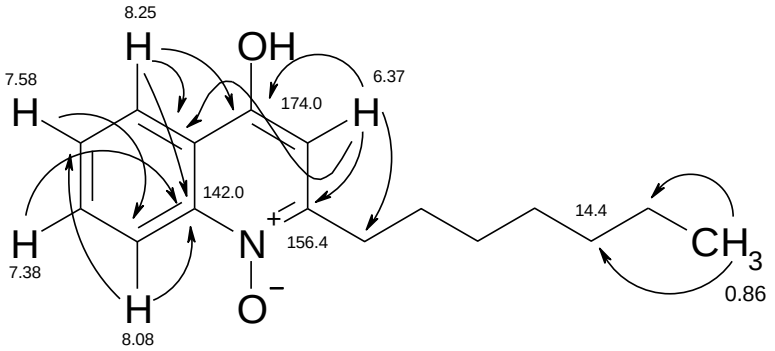


Figure 179: HMBC (→) correlations of KF8940 (**110**)

The ESI mass spectrum of compound **110** displayed signals at *m/z* 260 $[M+H]^+$, 282 $[M+Na]^+$ and 519 $[2M+H]^+$ in the positive mode, and *m/z* 258 $[M-H]^+$ and 517 $[2M-H]^+$ in the negative mode. HRESIMS established the molecular formula as $C_{16}H_{21}NO_2$ and confirmed the presence of one nitrogen atom in this compound. A search in AntiBase with these data gave KF8940 (**110**) as the result. It was further confirmed by the literature data.[158] KF8940 (**110**) was isolated from the culture broth of *Pseudomonas methanica* KY4634, which had inhibitory activity for the enzyme of 5-lipoxygenase of rat basophilic leukemia cells as well as from *Pseudomonas aeruginosa* [159] or by Jackson *et al.* as inhibitor for streptomycin [160], and by Lightbowjn *et al*. as inhibitor of cytochrome systems of heart and special bacteria.[161]

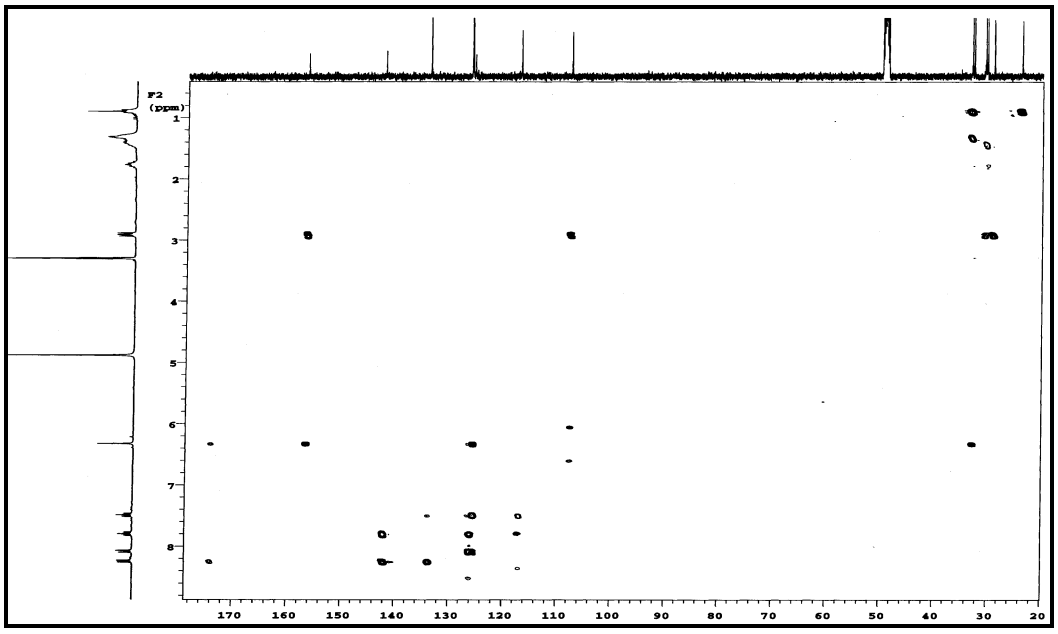
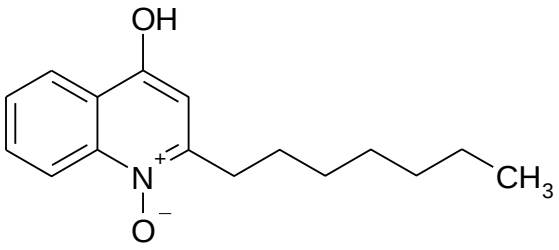


Figure 180: HMBC spectrum (CD_3OD, 500 MHz) of KF8940 (**110**)



110

4.13 Terrestrial *Streptomyces* sp. N859

The crude extract of the terrestrial *Streptomyces* sp. N859 showed no biological activity against the tested microorganisms, while the TLC analysis exhibited bands with different colour reactions with anisaldehyde/sulphuric acid. Ehrlich's reagent and chlorine/anisidine indicated the presence of indole derivatives as well as peptidic substances.

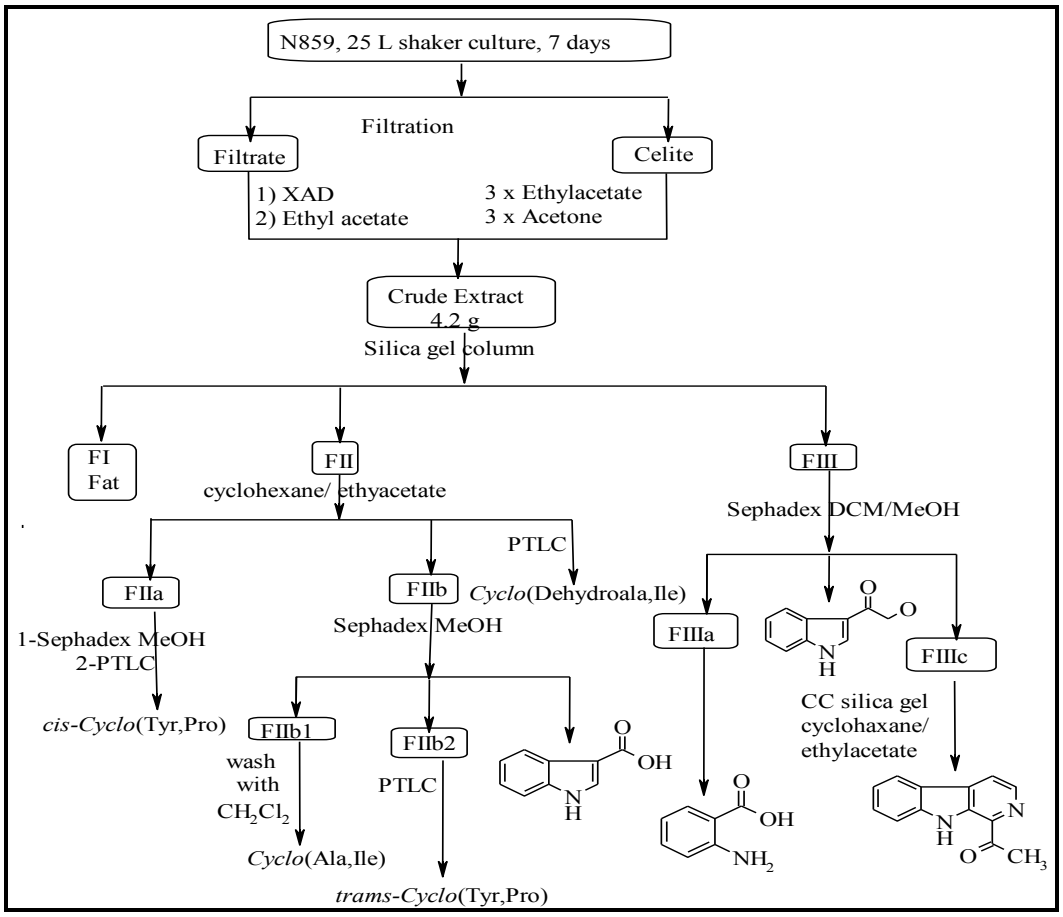


Figure 181: Work-up scheme for the terrestrial *Streptomyces* sp. N859.

4.13.1 1-Acetyl-*β*-carboline

Fraction III exhibited a middle polar blue fluorescent band, which showed a pale yellow colouration with Ehrlich's reagent and anisaldehyde/sulphuric acid. The constituent was purified on Sephadex LH-20 followed by silica gel eluted with cyclohexane/ethyl acetate to give a pure compound **111** as a pale yellow solid. The ^{1}H NMR spectrum of **111** showed broad singlet at δ 10.30 of an exchangeable proton, and two *ortho*-coupled signals at 8.54 (d, ^{3}J = 5.1 Hz, 1H, H-3) and 8.16 (d, 1H, ^{3}J = 5.1 Hz, H-4). The small coupling constant of these signals indicated to a heteroaromatic ring. In addition four multiplets at δ 8.15 (H-5), 7.62 (H-7,8) and 7.31 (H-6) were observed due to the presence of a 1,2-disubstituted aromatic ring. Finally the aliphatic region exhibited a singlet of an aromatic bound methyl group at δ 2.96, which was possibly present in *peri*-position to a carbonyl group, or may be present as NCH_3. The ESI mass spectra determined the molecular weight of **111** as 210 Dalton. A search in AntiBase led to 1-acetyl-*β*-carboline (**111**), which was further confirmed by comparing with the literature.[162]

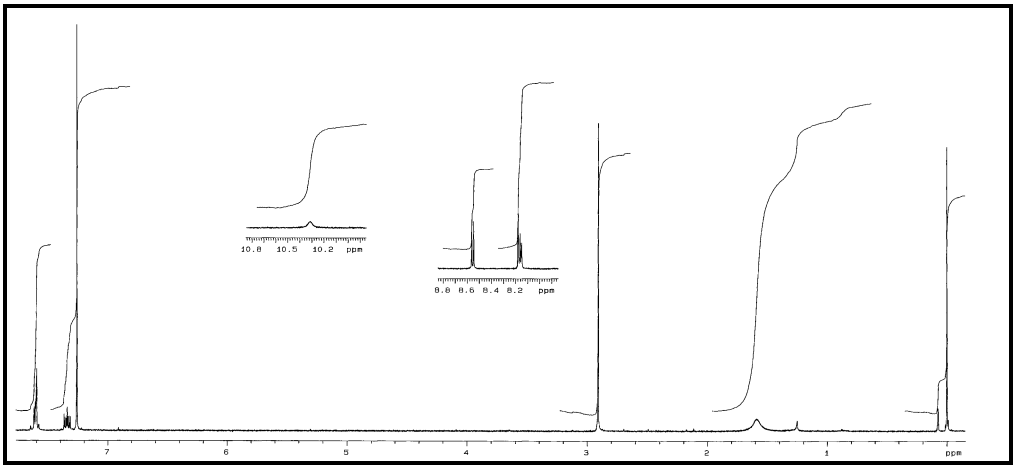
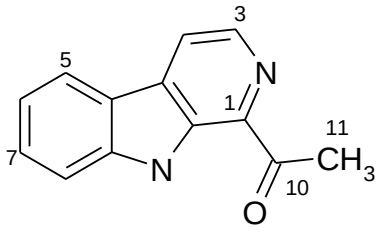


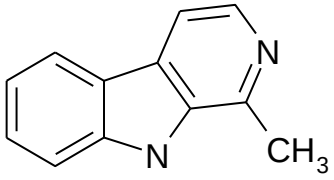
Figure 182: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 1-acetyl-*β*-carboline (**111**)

Compound **111** was isolated previously in our group by Huth.[163] *β*-Carboline alkaloids initially have been isolated from plants. It has been reported that these compounds are biologically active and possibly they play an important role as neuromodulators[164] and demonstrate hypnotic and antiepileptic activities.[165] 1-Acetyl-*β*-carboline (**111**) was isolated from the fern, *Hypodematium squamuloso-pilosum*,[162] the bark of *Ailanthus malabarica (*Simaroubaceae), and the sponge *Tedania ignis*.[166] Harman (**112**) is a 1-methyl-*β*-carboline alkaloid and was isolated from plants,[167]

fungi, microorganisms and marine animals.[168,169] It exhibited high pharmacological effects, e.g. by inhibiting the mononaminooxidase (MAO) and the cAMP-phosphodiesterase.[170]



111



112

4.13.2 *Cyclo*(**Dehydroala,Ile**)

Working up of fraction II led to the isolation of a colourless solid **113** that showed on the thin layer chromatogram a UV absorbing band at 254 nm and stained to orange with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **113** revealed two broad signals at δ = 10.47 and 8.40 corresponding to two exchangeable OH/NH protons. In the olefinic region two singlets at δ 5.18 (Ha-11) and 4.79 (Hb-11) each with integration of 1H were observed. Additionally, the aliphatic region exhibited a 2H methine signal at δ 3.96 (t, H-6) which could be connected to a heteroatom: a methine at δ 1.80 (m, H-8), a methylene group at δ 1.58 (m, H-7) as well as two methyl doublets at δ 0.86 (H-9, 10) indicated the presence of an isobutyl group.

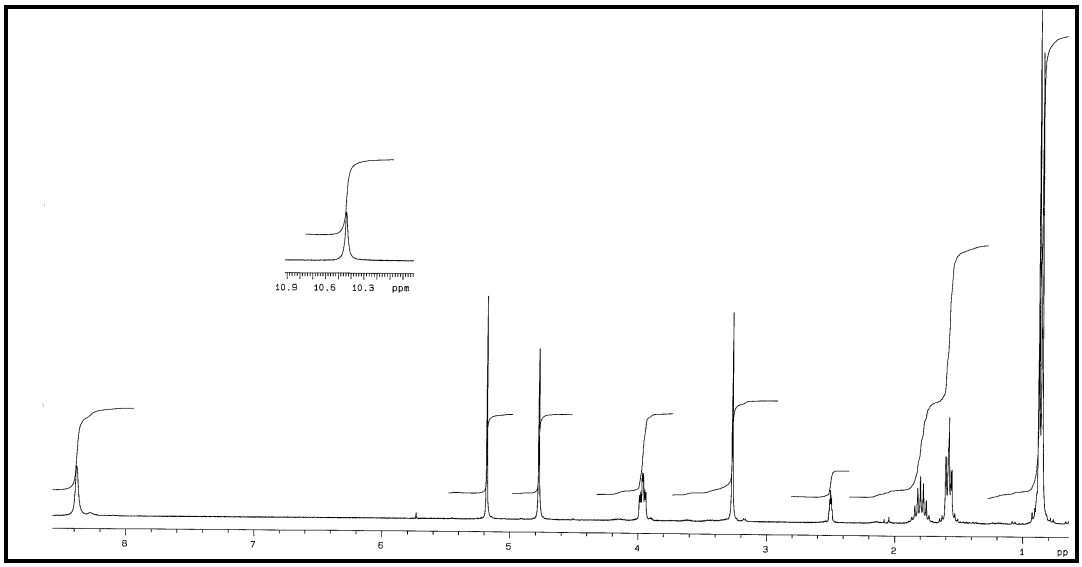


Figure 183: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of *cyclo*(Dehydroala,Ile) (**113**)

In the ^{13}C NMR spectrum there were two signals of amide carbonyls visible at δ 166.3 and 158.0, as well as two quaternary carbon signals at δ 134.5 and δ 98.8 Furthermore one methine group at δ 53.7 linked to a heteroatom, one methylene carbon signal at δ 43.5 and an isopropyl group at δ 23.4, 22.6, 22.1 were observed.

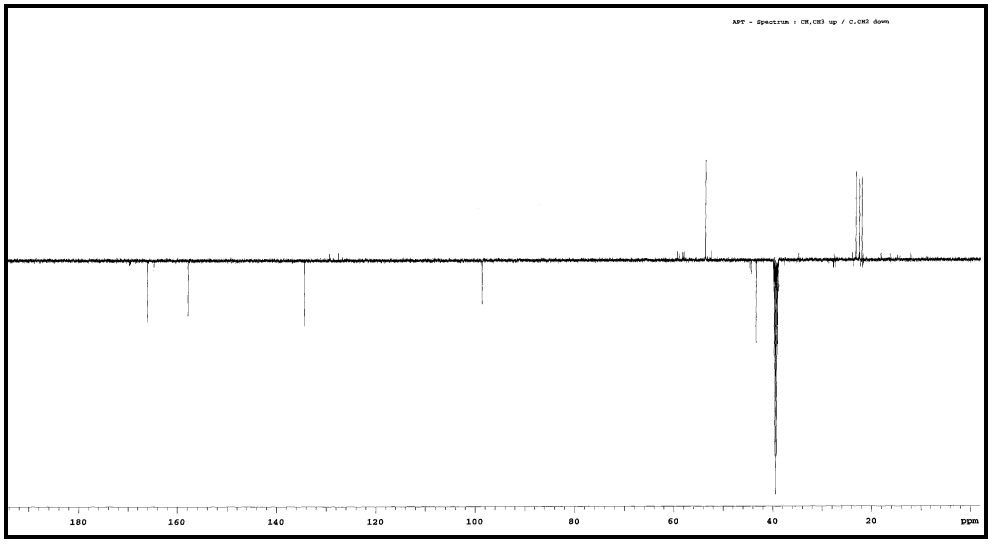
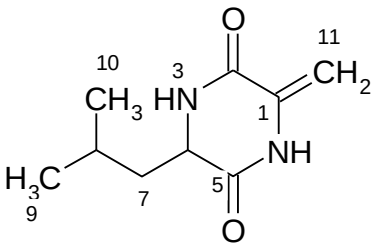


Figure 184: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of *cyclo*(Dehydroala,Ile) (**113**)

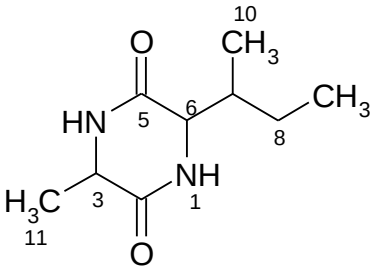
The molecular weight of compound **113** was deduced as 182.1 from the negative ESIMS, showing the molecular ion peak of *m*/*z* 385.0 [2M-2H+Na]-, which corresponded to the molecular formula of $C_9H_{14}N_2O_2$. Using the spectroscopic data of **113** and searching in AntiBase gave *cyclo*(Dehydroala-Ile) **113** as a result, which was further confirmed by comparison with the spectra from our group collection.[171] Compound **113** was isolated for the first time from *Penicillium* sp. F70614 and showed inhibition effect against α-glucosidase.[172]



113

4.13.3 *Cyclo*(**Ala,Ile**)

Fraction III was applied to Sephadex LH-20 followed by PTLC to afford compound **114** that turned to blue with chlorine/anisidine and violet when sprayed with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **114** contained three methine proton signals; two methines at δ 4.01, 3.91 were possibly connected to heteroatoms. A further methine signal was found at δ 1.86, and a methylene at δ 1.67 (m, H-8). Additionally, three methyls were seen at δ 1.44 (d, H-11), 0.97 (d, H-10) and 0.96 (t, H-9). The ESI mass spectrum showed molecular ion peaks at *m/z* $[2M+Na]^+$ and 241.0 $[M+Na]^+$ which fixed the molecular weight as 185 Dalton. Compound **114** was found to have a molecular formula of $C_9H_{16}N_2O_2$ by HRESIMS. A search in the AntiBase using all of the spectroscopic data above led to *cyclo*(Ala,Leu) (**114**) as a result. *Cyclo*(Ala,Ile) (**114**) was previously isolated from the marine-derived *Streptomyces* sp. 3320 [173], the Antarctic psychrophilic bacterium *Pseudoalteromonas haloplanktis* TA125,[174] *Aspergillus fumigatus* CY018, roots of *Panax notoginseng*,[175] and roots of *Panax notoginseng*.[176]



114

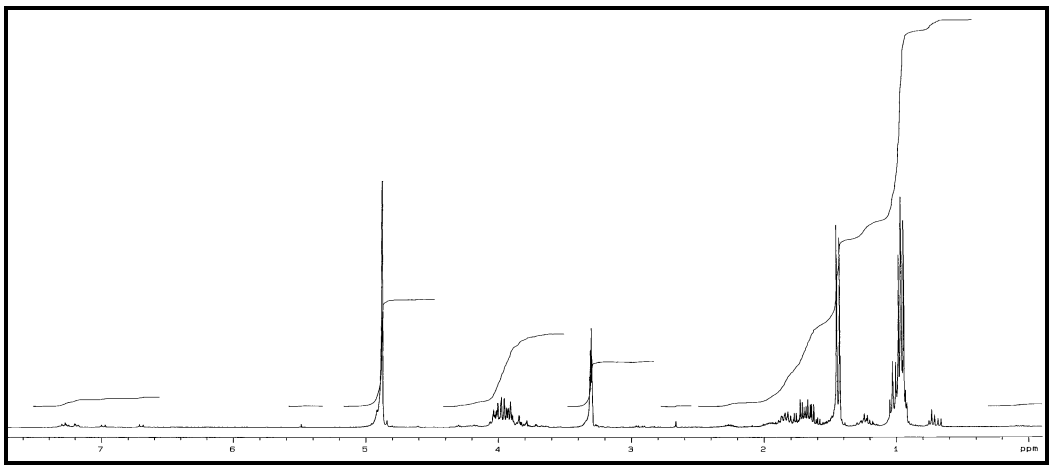
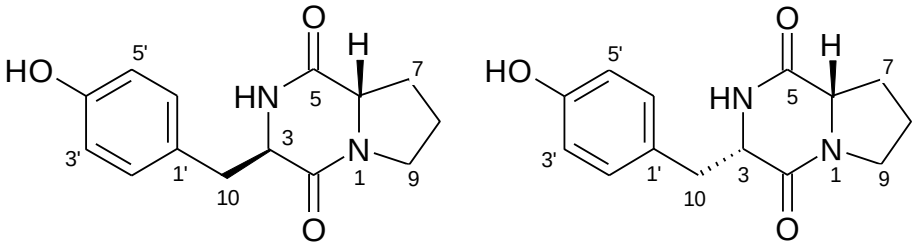


Figure 185: ^{1}H NMR spectrum (CD_3OD, 300) of *cyclo*(Ala,Ile) (**114**)

4.13.4 *Trans-Cyclo*(**Tyr,Pro**)

Compound **115** was found in fraction II as an UV absorbing zone, which stained to violet with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of compound **115** showed two *ortho*-coupled signals at δ 6.97 (H-2', 6') and 6.71 (H-3', 5'), which pointed to an AA',BB' system of an 1,4-disubstituted aromatic ring, as well as two signals at δ 4.35 and 4.03 for two methines attached to electron withdrawing substituents. The spectrum of **115** showed also two doublet of doublet signals for an ABX system of a methylene group at δ 3.09 and 2.87 (CH_2-10), as well as three methylene multiplets (CH_2-9) (δ 3.30, 2.62) attached to a heteroatom and CH_2-7,8 (δ 2.04, 1.84, 1.63). The ESI mass spectra determined the molecular weight of **115** as 260 Dalton by (+)-ESI and (-)-ESI modes. A search in AntiBase[77] with the aid of the spectroscopic data gave the two stereoisomeres, *trans-cyclo*(Tyr,Pro) (**115**) and *cis-cyclo*(Tyr,Pro) (**116**). The structure was further confirmed as *trans-cyclo*(Tyr,Pro) (**115**) by comparison with an authentic sample and spectra from our collection.



115 **116**

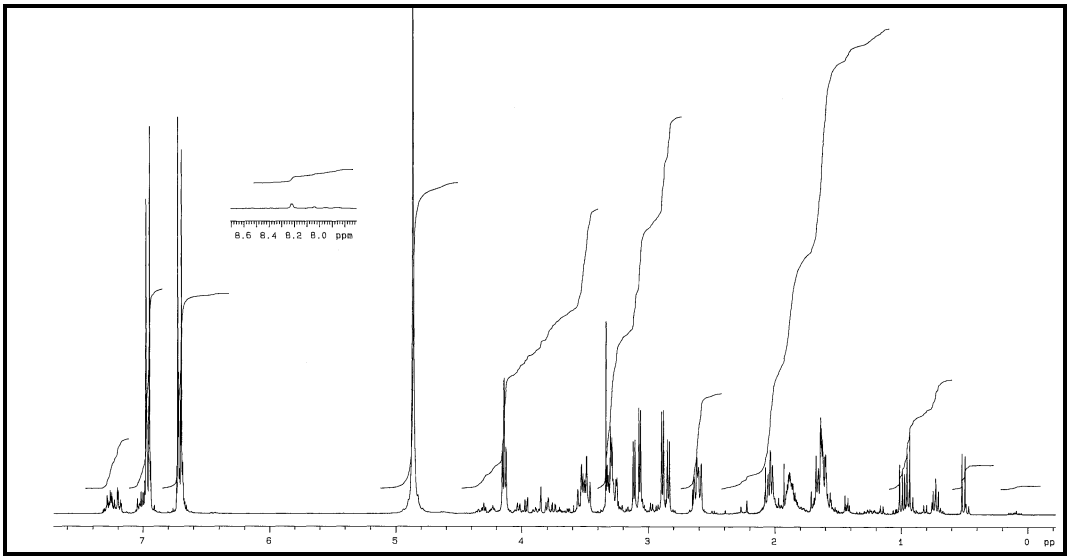


Figure 186: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *trans-Cyclo*(Tyr,Pro) (**115**)

4.13.5 *Cis-Cyclo*(Tyr,Pro)

Compound **116** was isolated from the same fraction as a colourless solid and showed similar physical and chemical behaviour with anisaldehyde/sulphuric acid and chlorine/anisidine reagent, pointing to a related structural analogue. The ^{1}H NMR spectrum showed very close similarity with compound **115**, showing signals of a 1,4-disubstituted benzene ring at δ 7.03 and 6.69, two methines at δ 4.35 and 4.03, as well as four methylene multiplets between δ 3.60-1.22. The negative ESIMS spectrum of compound **116** displayed *pseudo*molecular ion peaks at *m*/*z* 565, 542 and 283 corresponding to [2M-H+2Na], [2M+Na] and [M+Na] respectively, which fixed the molecular weight as 260 Dalton. *Cyclo*(Tyr,Pro) (**116**) was isolated from the terrestrial isolate of *Penicillium striatisporum*,[177] *Aspergillus flavipes*,[178] the sponge *Tedania anhelans*,[179] a marine-derived fungus *Chromocleista* sp.[180] and the fungus *Alternaria alternata*.[181] It was reported that *cyclo*(Tyr,Pro) (**116**) has pesticidal activity and inhibits germination.[182]

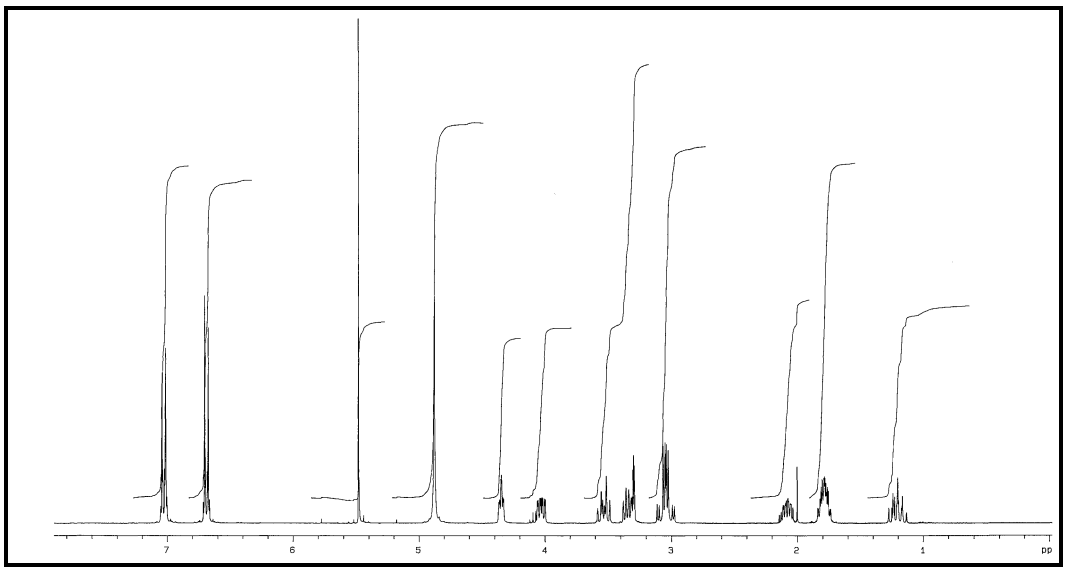


Figure 187: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of *cis-cyclo*(Tyr-Pro) (**116**)

4.13.6 3-Hydroxyacetylindole

Fraction III showed a UV absorbing band, which stained to orange with anisaldehyde/sulphuric acid and gave **117** after purification. The ^{1}H NMR spectrum showed a broad signal at δ 8.94 for an acidic proton, as well as signals at δ 8.28 (m, H-4), 7.47 (m, H-7) and 7.34 (m, H-5, 6) characteristic of a 1,2-disubstituted benzene ring, and one doublet at δ 7.93 (d, H-2) as well as an oxymethylene signal at δ 4.79 (H-9). The

molecular weight was determined as 175 Dalton, based on the EI mass spectrum. A search in AntiBase gave 3-hydroxyacetylindole (**117**). This was further confirmed by comparison with the authentic spectrum and the literature.[183,152]

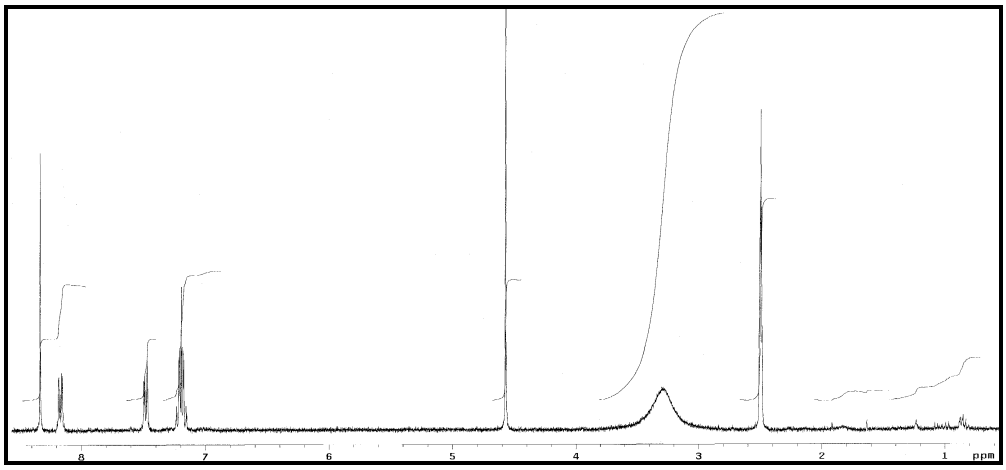
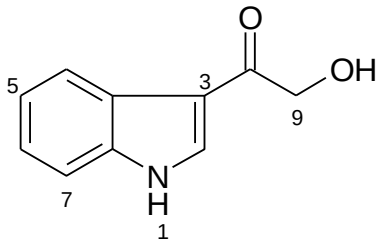


Figure 188: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of 3-hydroxyacetylindole (**117**)



117

4.14 Marine derived *Streptomyces* sp. B7547

The crude extract of the marine derived *Streptomyces* sp. B7547 showed strong biological activity against the tested microorganisms, Figure 257. The TLC analysis exhibited different colour reactions with anisaldehyde/sulphuric acid and gave a violet colour with sodium hydroxide, indicating of *peri*-hydroxyquinone.

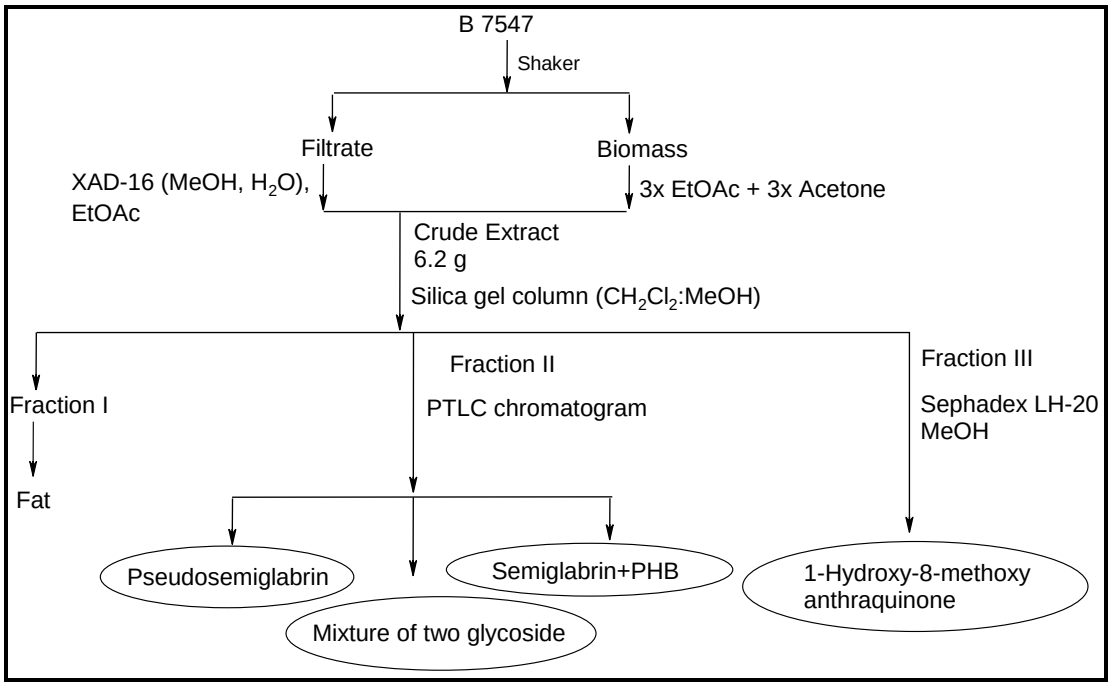


Figure 189: Work-up scheme of the marine-derived *Streptomyces* sp. B7547

4.14.1 Pseudosemiglabrin

Pseudosemiglabrin (**118**) was isolated by PTLC as a white powder, which showed a blue fluorescent zone and gave a blue-green colour with anisaldehyde/sulphuric acid. In the aromatic region two *ortho*-coupled aromatic protons were observed at δ 8.04 (H-5) and 6.94 (H-6), as well as doublets of a doublet at δ 7.93 (H-2', H-6') with the intensity of two protons. In addition three aromatic proton signals overlapping at δ 7.58- 7.49 (H-3', H-5', H-4'), a pattern indicative of a benzene ring, and a singlet at δ 6.80 (H-3) were observed. In the olefinic region three methine protons appeared as doublets at δ 6.50 (H-2''), 5.60 (H-3''') and doublets of a doublet at δ 4.74 (d, H-3''), which was connected to an sp^2 carbon. In the upfield region methyl signals were observed at δ 1.35 and 1.08 and assigned to H_3-4''' and H_3-5''', respectively. An acetyl signal was also observed at δ 1.42.

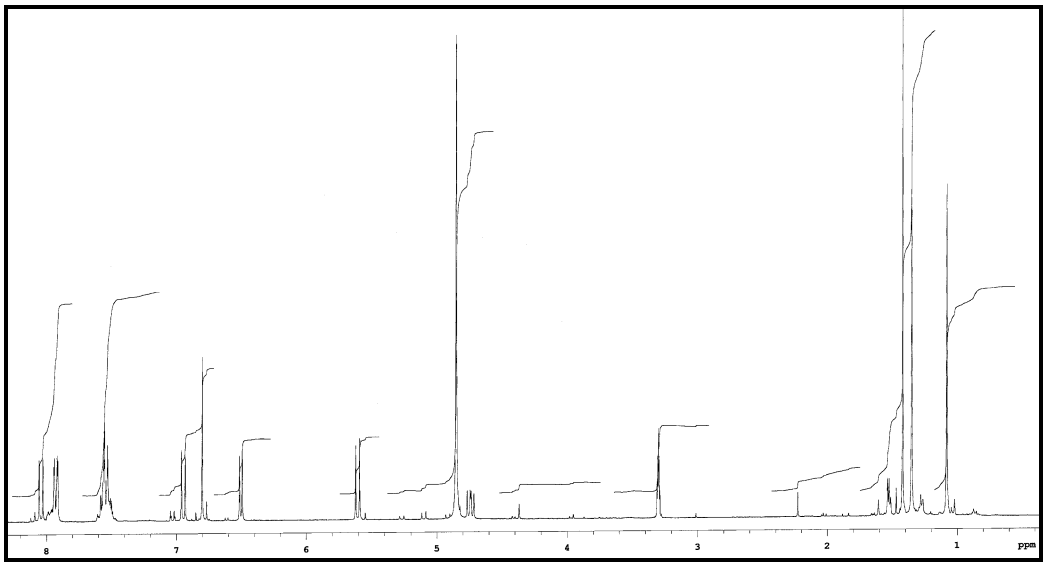


Figure 190: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of pseudosemiglabrin (**118**)

The ^{13}C NMR spectrum showed the presence of one ketone carbonyl at δ 179.6, three oxygenated sp^2 carbon signals at δ 166.3 (C-7), 164.8 (C-2) and 155.2 (C-9) as well as further 11 sp^2 signals between δ 133.0 - 107.5 to establish altogether a flavone moiety. Three methine carbon signals were assigned to an acetal carbon at δ 113.7 (C-2"), an oxygenated methine at δ 78.2 (C-3"') and another one connected to an sp^2 carbon at δ 49.0 (C-3"). In addition, there were three methyl signals assigned to C-4"' (δ 27.8), C-5"' (δ 23.6), and an acetyl group at δ 20.4 were observed.

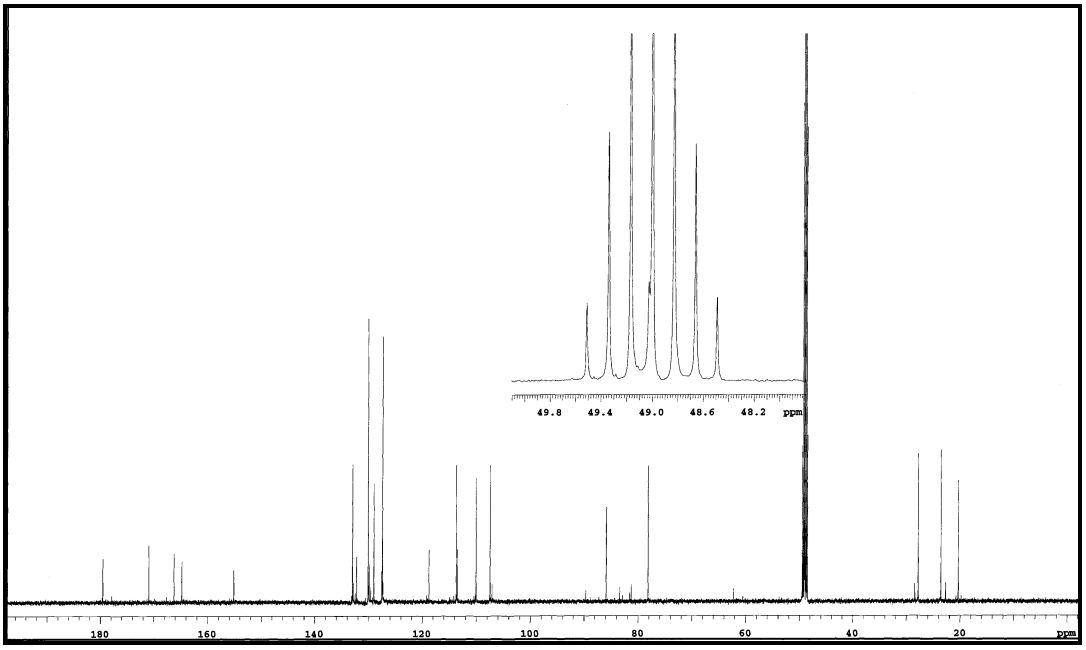


Figure 191: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of pseudosemiglabrin (**118**)

2D experiments (COSY and HMBC) exhibited all correlations. A proton singlet at δ 6.8 showed strong correlation to quaternary carbons at δ 132.2 and 179.6 (the latter for the carbonyl of a flavonoid moiety), and also to the quaternary carbon at δ 118.8. From the COSY spectrum the doublet at δ 6.50 (suggested to be due to a hemiacetal proton) showed strong correlation to a multiplet at δ 4.64 that showed ^3J correlation to another oxymethine proton at δ 5.60. This proton showed strong correlation to an acetyl carbon at δ 170.9 and a quaternary carbon at δ 85.6, which displayed strong correlation from dimethyl protons at δ 27.8 and 23.6.

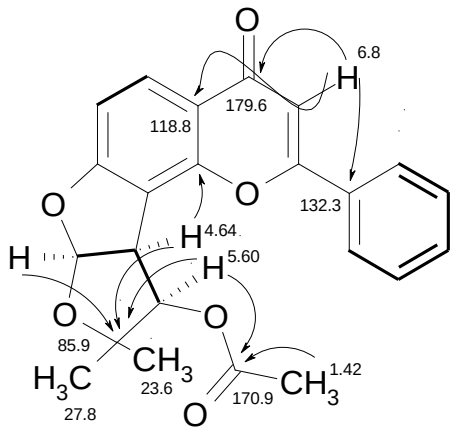


Figure 192: ^{1}H,^{1}H COSY (▬) and HMBC (→) couplings of pseudosemiglabrin (**118**)

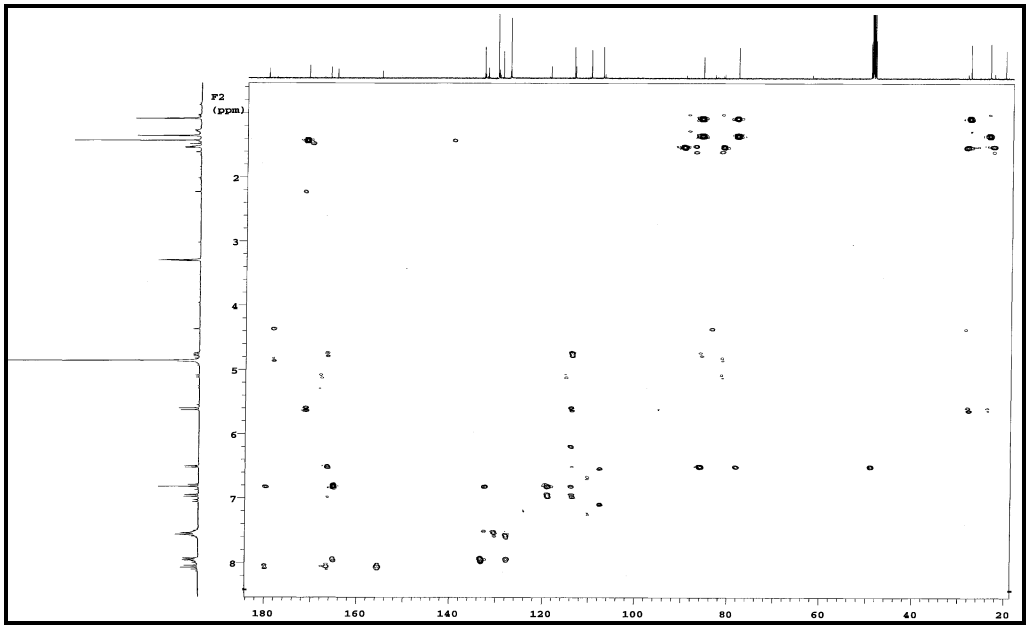


Figure 193: HMBC spectrum (CD_3OD, 500 MHz) of pseudosemiglabrin (**118**)

A search in AntiBase supported by ^{1}H, ^{13}C NMR, 2D and MS spectroscopic data led to pseudosemiglabrin (**118**).[184,185] The result was further confirmed by the literature data [186] and comparing with authentic spectra.

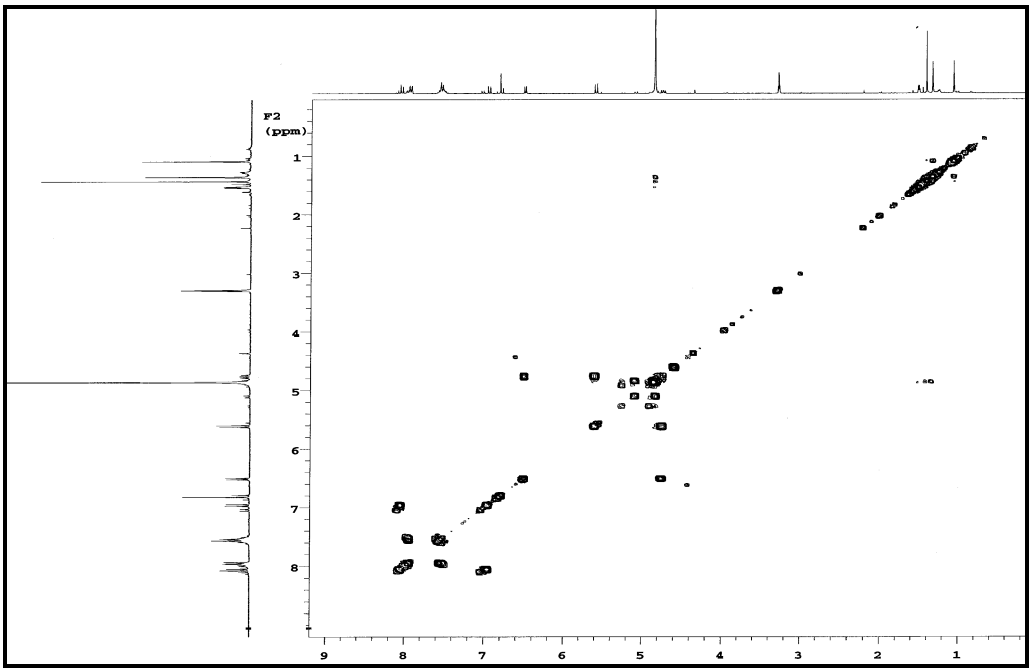
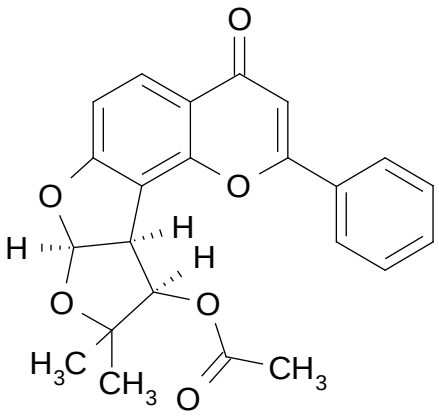


Figure 194: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 500 MHz) of pseudosemiglabrin (**118**)



118

Pseudosemiglabrin (**118**) isolated from medicinal plants of the genus *Tephrosia*.[187] It was isolated along with semiglabrin from *Tephrosia semiglabra* with *in vitro* inhibition effects on human platelet aggregation,[188] isolated from *Tephrosia purpurea* and from *Tephrosia nubica*.[189]

4.14.2 1-Hydroxy-8-methoxy anthraquinone

1-Hydroxy-8-methoxy anthraquinone (**119**) was isolated as yellow crystals characterized by the violet colour reaction with sodium hydroxide solution indicative of a *peri*-hydroxyquinone moiety. The ^{1}H NMR spectrum of **119** revealed a downfield 1H singlet of a chelated hydroxyl group at δ 12.93. In the aromatic region six proton signals were observed: the doublet of doublet at δ 7.95 (J = 7.7, J = 1.0), two overlapped 2H triplets at δ 7.74, a triplet at δ 7.60 (J = 8.2), doublet at δ 7.35 (J = 8.4), and finally doublets of a doublet at δ 7.27 (J = 8.3, J = 1.2) were observed. The coupling pattern and the coupling constants indicated two 1,2,3-trisubstituted benzene rings. In addition, a singlet of an sp^2 bound methoxy group at δ 4.06 was exhibited.

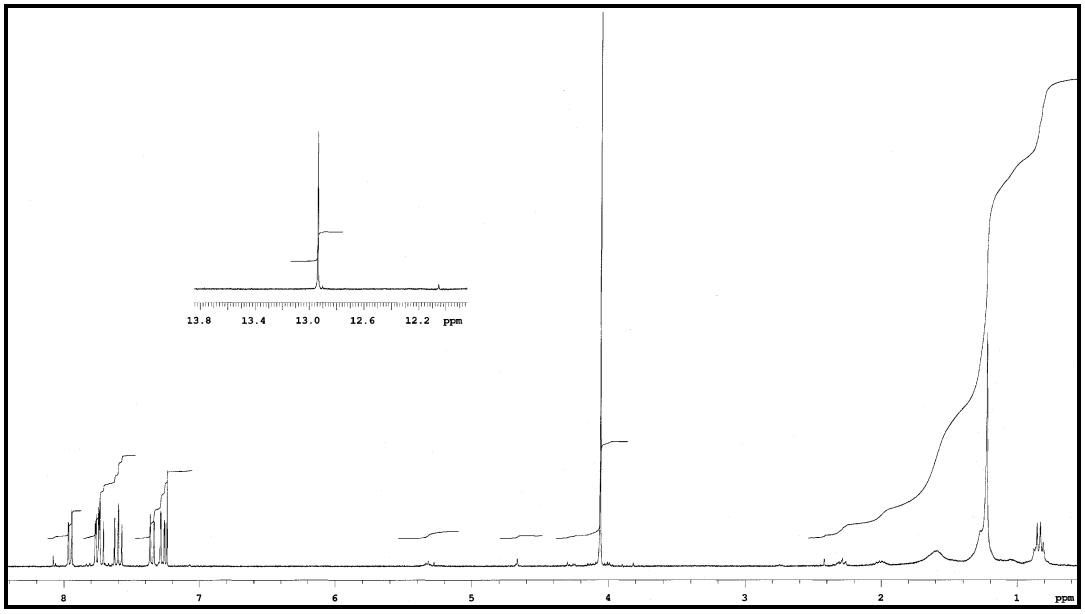


Figure 195: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of 1-hydroxy-8-methoxy anthraquinone (**119**)

The ^{13}C NMR spectrum revealed 15 carbons, among them two signals at δ 188.7, 182.7 for carbonyls of an anthraquinone system, two quaternary oxygenated carbons at δ 162.5, 160.7, six methine sp^2 carbons at δ 135.8, 135.7, 124.7, 120.7, 118.8, 118.1, in addition to four quaternary carbons in the sp^2 region. Finally, a methoxy carbon at δ 56.7 was determined according to the HSQC spectrum.

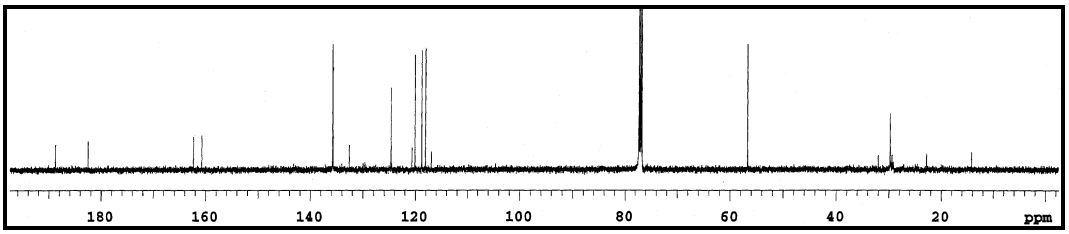
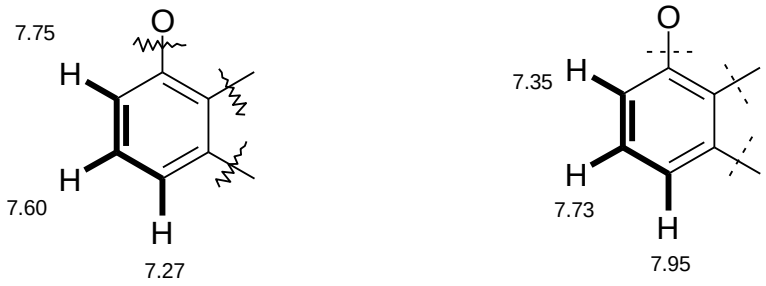


Figure 196: ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of 1-hydroxy-8-methoxy anthraquinone (**119**)

The $^{1}H,^{1}H$ COSY spectrum showed two partial structures: In fragment **A** correlated a doublet at δ 7.75 with a triplet proton at δ 7.60 which showed cross signals to the doublet at δ 7.27. In addition, the doublet at δ 7.35 displayed a ^{3}J correlation to the triplet at δ 7.73 that exhibited strong three bonds coupling to the doublet at δ 7.95 (fragment **B**).



A **B**

Figure 197: $^{1}H,^{1}H$ COSY fragments (▬) of 1-hydroxy-8-methoxyanthraquinone (**119**)

From the HMBC spectrum the proton at δ 7.60 showed a ^{3}J coupling with the quaternary carbon at δ 162.3, and also showed ^{2}J with the quaternary carbon at δ 132.6. The proton at δ 7.27 showed ^{3}J coupling with the carbonyl at δ 182.7 and 117.0, while the chelated proton at δ 12.97 correlated to the quaternary carbon at δ 162.5 and strong correlation to the methine at δ 118.8 and the quaternary carbon at δ 117.0. In addition, the methoxy protons at δ 4.1 exhibited correlation to quaternary carbon at δ 160.7, and the doublet at δ 7.97 correlated to the quinone carbonyl at δ 182.7 and to the quaternary carbon at δ 118.1. All these data confirmed the structure of 1-hydroxy-8-methoxyanthraquinone (**119**).

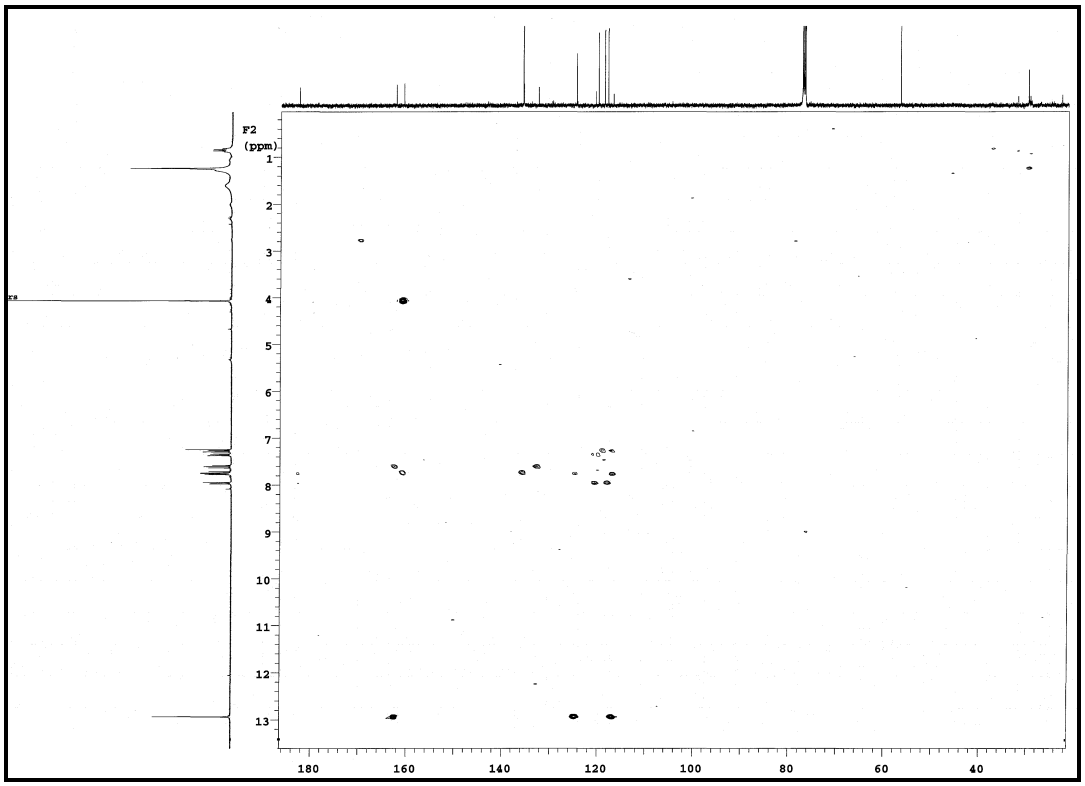


Figure 198: HMBC spectrum ($CDCl_3$, 500 MHz) of 1-hydroxy-8-methoxyanthraquinone (**119**)

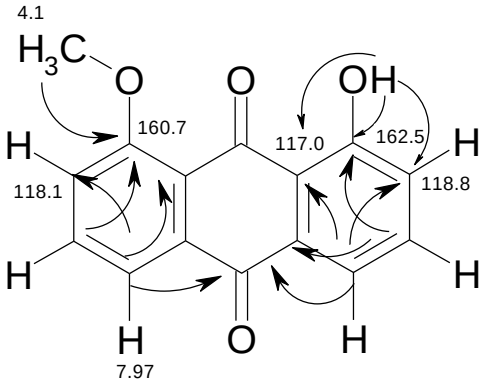
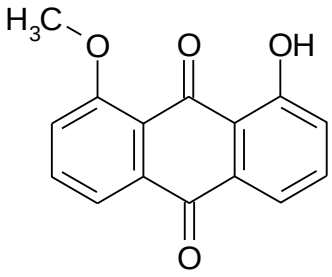


Figure 199: HMBC (→) couplings of 1-hydroxy-8-methoxyanthraquinone (**119**)

A search in AntiBase[77] supported by the ^{1}H, ^{13}C NMR, and HMBC spectroscopic data led to 1-hydroxy-8-methoxyanthraquinone (**119**) The result was further confirmed by the literature data.[190] 1-Hydroxy-8-methoxyanthraquinone (**119**) was isolated for first time from the fungus *Leptographium wageneri* by Ayer *et al*. and was synthesised by Tanaka [191] and Berger.[192]



119

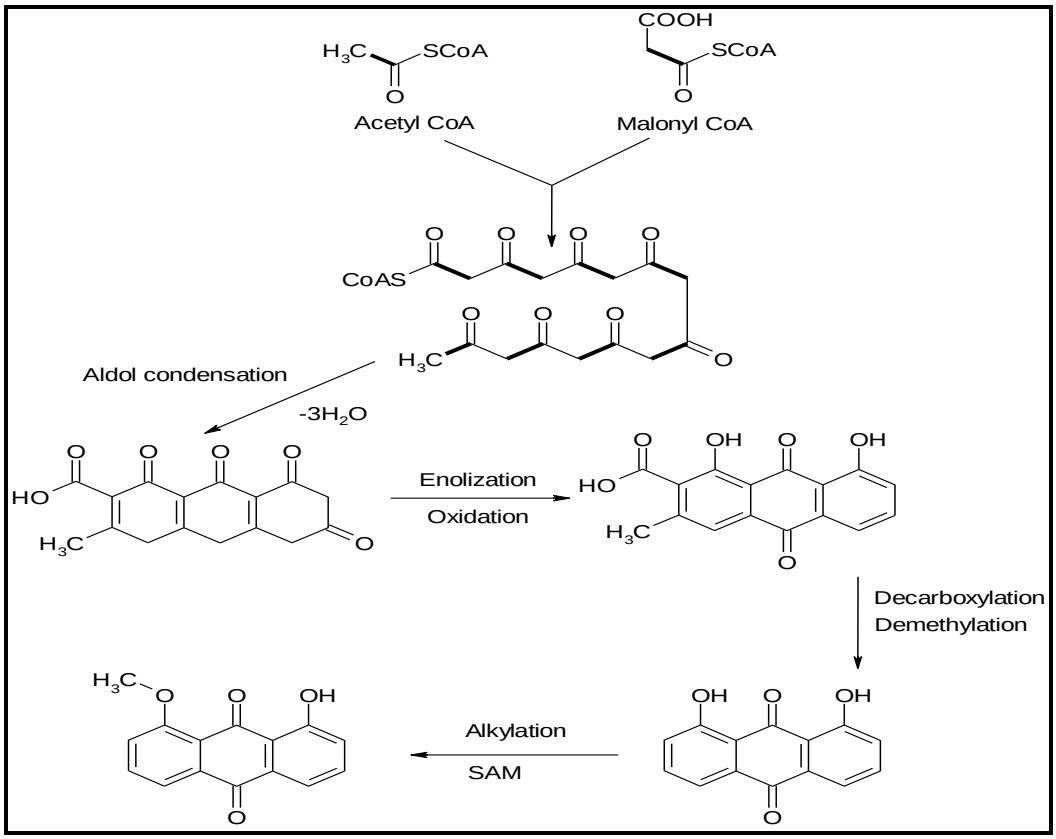


Figure 200: Proposed biosynthetic pathway of 1-hydroxy-8-methoxy anthraquinone

4.14.3 Mixture of two glycosides

A mixture of two isomeric 2-oxopropyl-α-D-glucopyranosides (**120**) was isolated as colourless oily substance, which gave a brown coloured zone with anisaldehyde reagent and heating. The molecular weight was determined based on the ESI mass spectrum, which showed ion peaks at *m/z* 305 $[M-H]^-$ and 611 $[2M-H]^-$, and in positive mode ions at *m/z* 329 $[M+Na]^+$ and 635 $[2M+Na]^+$, corresponding to the molecular weight of 306 Dalton. HRESIMS established the molecular formula as $C_{12}H_{18}O_9$. The ^1H NMR spectrum of **120** revealed in the olefinic region a multiplet

signal at δ 6.61 and an anomeric proton at δ 5.31. In the aliphatic region, an ABX system appeared at δ 4.75 and 4.28, which suggested a methylene group attached to oxygen, while in the region at δ 4.28-3.29 nine protons of oxymethines and methylenes overlapped. In addition a methyl singlet at δ 1.52 was visible. A search in AntiBase supported by above spectroscopic data led to the identification of a mixture of two epimers. It was further confirmed by the literature data.[193]

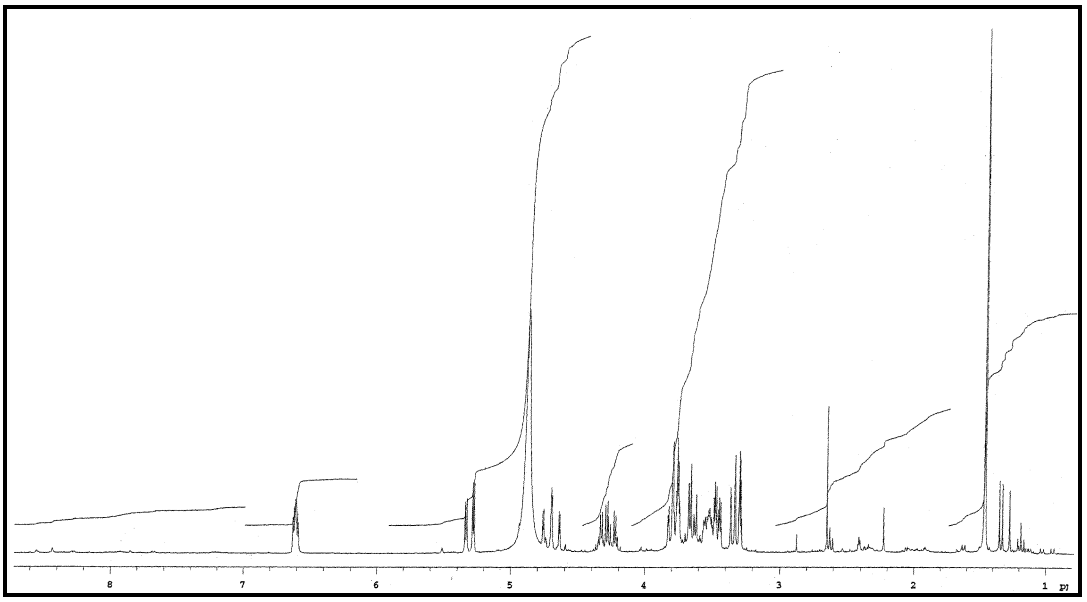
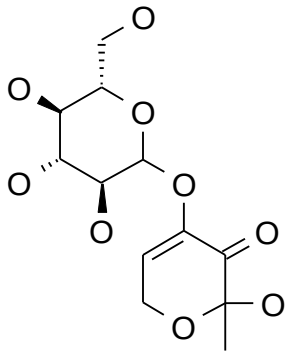


Figure 201: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of mixture of glycosides (**120**)



120

4.15 Terrestrial *Streptomyces* sp. GW 7/186

The crude extract of the terrestrial *Streptomyces* sp. GW 7/186 showed good biological activity against the tested microorganisms, see Figure 258, and the TLC analysis exhibited different colour reactions with anisaldehyde/sulphuric acid.

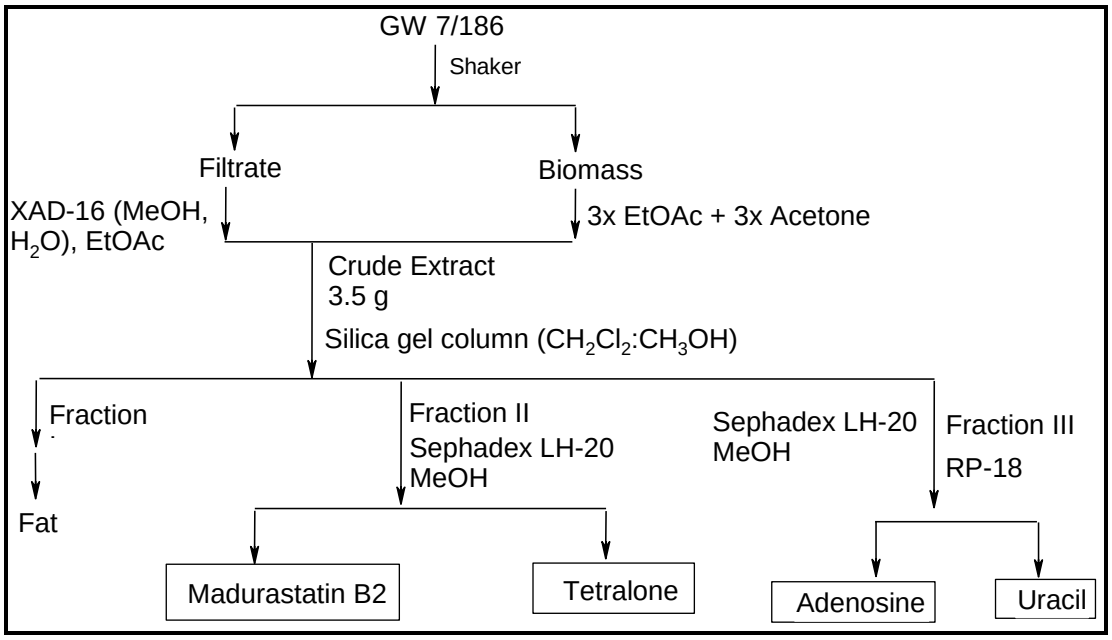


Figure 202: Work-up scheme of the terrestrial *Streptomyces* sp. GW 7/186

4.15.1 Madurastatin B2

Madurastatin B2 (**121**) was isolated as a colourless solid from fraction II, which was UV active and turned blue by spraying with anisaldehyde/sulphuric acid and heating. ESIMS showed *pseudo*molecular ions at *m/z* 224 $[M-H]^+$ and 248 $[M+Na]^+$ corresponding to a molecular weight of 225 Dalton. The odd mass number was an indication of an odd number of nitrogen atoms in the molecule. HRESIMS established the molecular formula as $C_{10}H_{10}N_1O_5$ and confirmed the presence of nitrogen in compound **121**. The ^1H NMR spectrum exhibited in the aromatic region two dd signals and two triplets of a 1,2-disubstitued benzene ring. In the aliphatic region one methine proton at δ 4.06 attached to heteroatom was seen, as well as methylene protons of an ABX system at δ 3.70 and 3.54. Their downfield shift indicated they were in connection with sp^2 carbons or a heteroatom.

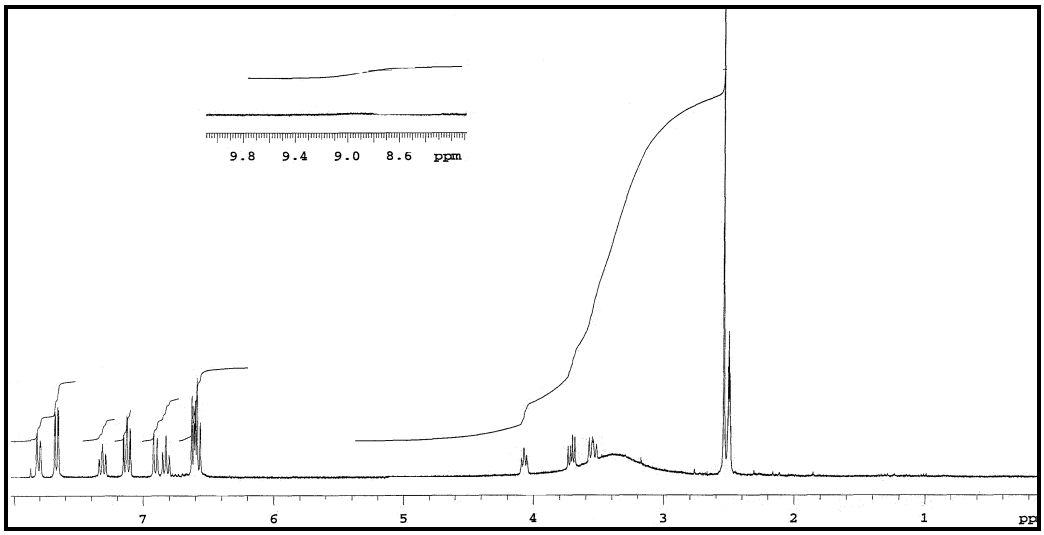


Figure 203: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of madurastatin B2 (**121**)

The ^{13}C NMR spectrum exhibited 10 carbon signals, among of them two carbonyl groups of amides, acids or esters at δ 172.9, 166.5. Additionally a quaternary oxygenated carbon at δ 158.9, and four methine carbons in the aromatic region at δ 132.6, 128.5, 117.9, 117.1 were present. A methylene group was observed at δ 62.5, and a methine carbon at δ 55.5.

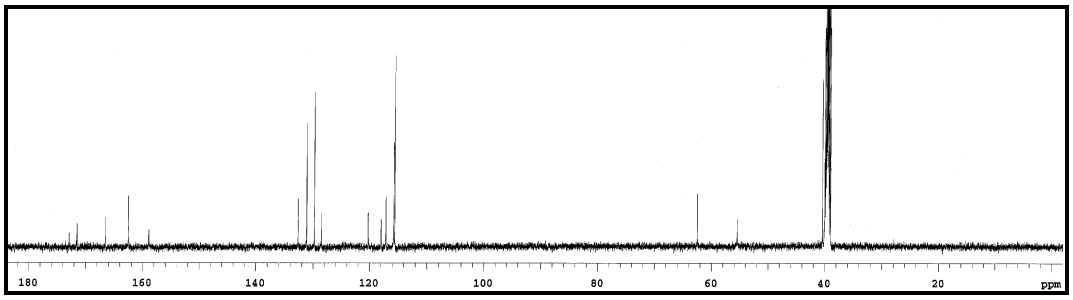
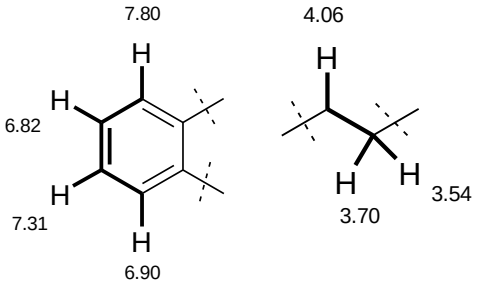


Figure 204: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of madurastatin B2 (**121**)

The ^{1}H,^{1}H COSY spectrum revealed three bond correlations from the doublet at δ 7.80 (CH-6) to a triplet at δ 6.82. The latter displayed a ^{3}J correlation to a triplet at δ 7.31, which showed strong coupling to another doublet at δ 6.90 to confirm a benzene ring (fragment **A**). Additionally the COSY spectrum displayed a methine signal at δ 4.06 (CH-9), which showed a cross link to the methylene signals at δ 3.70, 3.54 (fragment **B**).



A **B**

Figure 205: ^{1}H,^{1}H COSY (▬) couplings of madurastatin B2 (**121**)

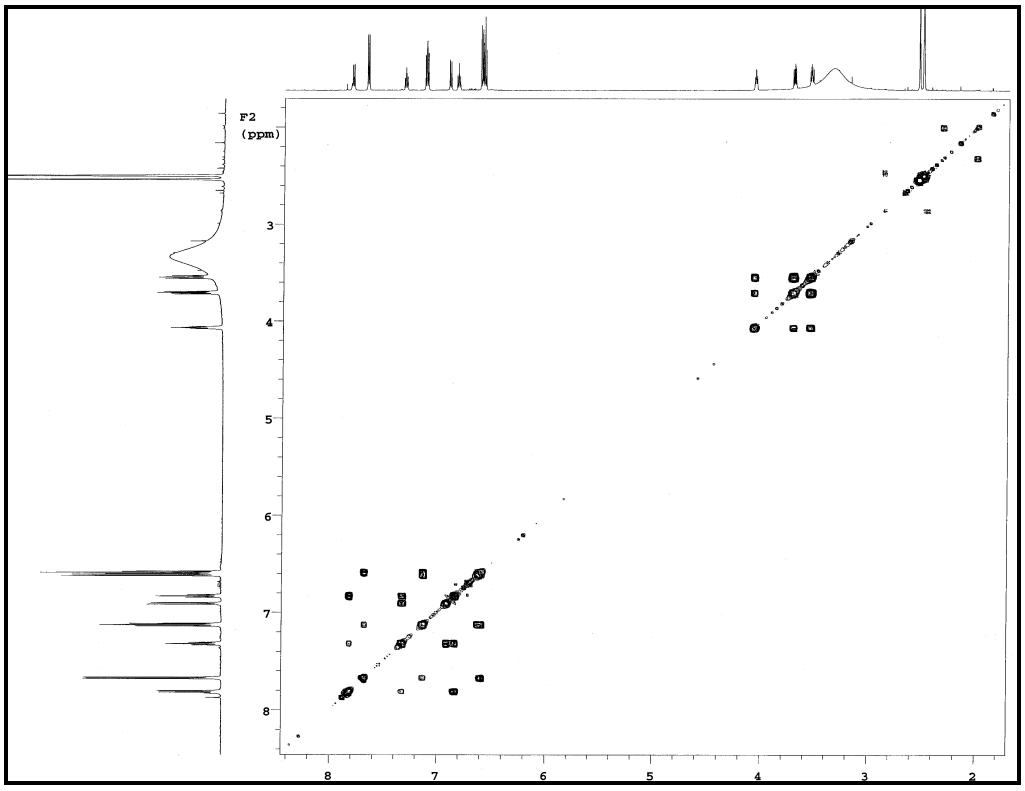
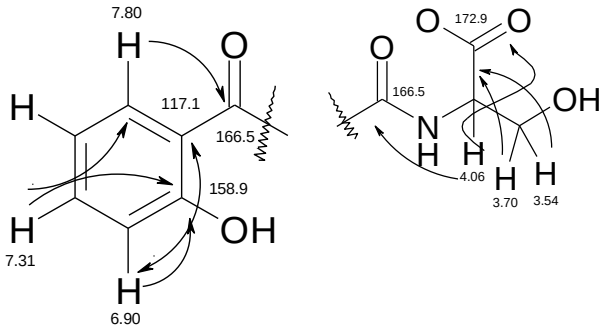


Figure 206: ^{1}H,^{1}H COSY spectrum (CD_3OD, 500 MHz) of madurastatin B2 (**121**)

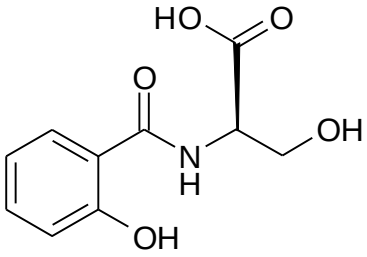
The HMBC spectrum displayed strong correlations from the triplet at δ 7.31 to the doublet at δ 128.5; the quaternary carbon at δ 158.9 showed also a correlation from the signal at δ 7.80. The latter proton exhibited three-bond correlation to the carbonyl at δ 166.5, which showed strong correlation from the methine proton at 4.06 along the amide bond. The methine proton showed also strong correlation to another acid carbonyl at δ 172.9 and was supported by another correlation from the methylene protons at δ 3.70 and 3.54.



A **B**

Figure 207: HMBC (→) couplings of madurastatin B2 (**121**)

A search in AntiBase with these data gave madurastatin B2 (**121**) as a result. It was further confirmed by the literature data.[194] Madurastatin B2 (**121**) has been recently cyclized to an oxazoline, which had activity against *Mycobacterium tuberculosis*.[195] A contamination was identified as anthranillic acid by shift values and 2D correlations, but could not be separated.



121

4.15.2 4*β*,8-Dihydroxy-3α-hydroxymethyl-4α-methyl-1,2,3,4-tetrahydronaphthalen-1-one

The ^{1}H NMR spectrum of **122** exhibited in the aromatic region three 1H signals at δ 7.50 (t), 7.16 (d), and 6.80 (d). This ABC pattern allowed the construction of a 1,2,3-trisubstituted benzene ring. In the aliphatic region methylene protons of an ABX system were observed at δ 3.87 and 3.43; their downfield shift indicated a connection with sp^2 carbons or heteroatoms. Furthermore another methylene group at δ 2.91 was displayed as well as a methine multiplet at δ 2.36. Finally a methyl singlet at δ 1.60 was exhibited.

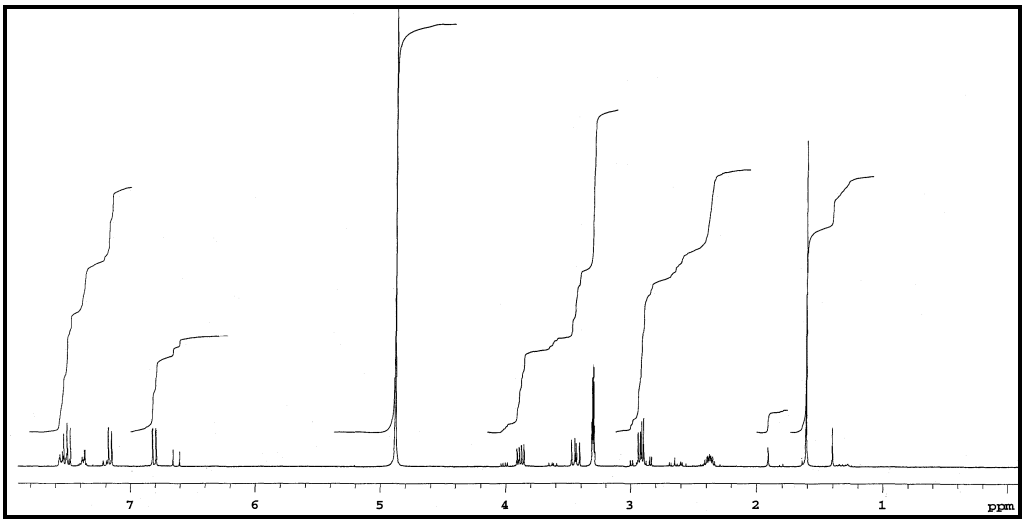
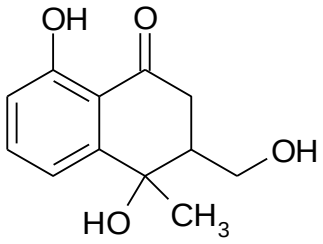


Figure 208: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of compound **122**

The ESI mass spectrum of compound **122** displayed signals at *m/z* 245 $[M+Na]^+$, 421 $[2M-H]^-$. According to these spectroscopic data and a search in AntiBase this compound was determined as 4β,8-dihydroxy-3α-hydroxymethyl-4α-methyl-1,2,3,4-tetrahydronaphthalen-1-one (**122**). The assignment confirmed by literature data [196] and authentic spectra.



122

4.16 Terrestrial *Streptomyces* sp. MH4

The terrestrial *Streptomyces* sp. MH4 was selected according to the chemical and biological screening. Antimicrobial activity of the crude extract against different microorganisms is summarized in Table 25. On TLC the crude extract showed two UV absorbing bands of middle polarity, which turned to blue and red colours, respectively, with anisaldehyde/sulphuric acid.

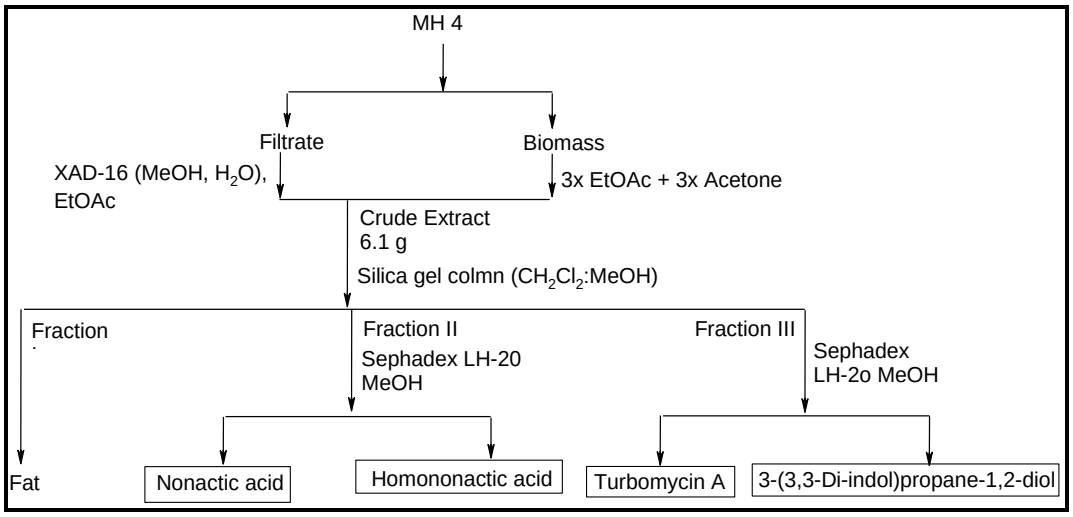


Figure 209: Work-up scheme of terrestrial *Streptomyces* sp. MH4

4.16.1 Nonactic acid

Nonactic acid (**123**) was isolated as oily substance from a UV-inactive fraction, which turned blue with anisaldehyde reagent. The ^{1}H NMR spectrum of **123** exhibited signals for 18 protons in the aliphatic region: 2H overlapped at δ 3.99, 1H multiplet appeared at δ 3.89, and these three protons were obviously attached to heteroatoms. Furthermore a 1H triplet at δ 2.42 (^{3}J = 13.4, ^{4}J = 6.8) as well as a 2H multiplet at δ 1.99 was observed. Additionally a 4H multiplet at δ 1.58 and finally two methyl doublets at δ 1.15 (J = 6.2) and 1.09 (J = 6.9) were visible.

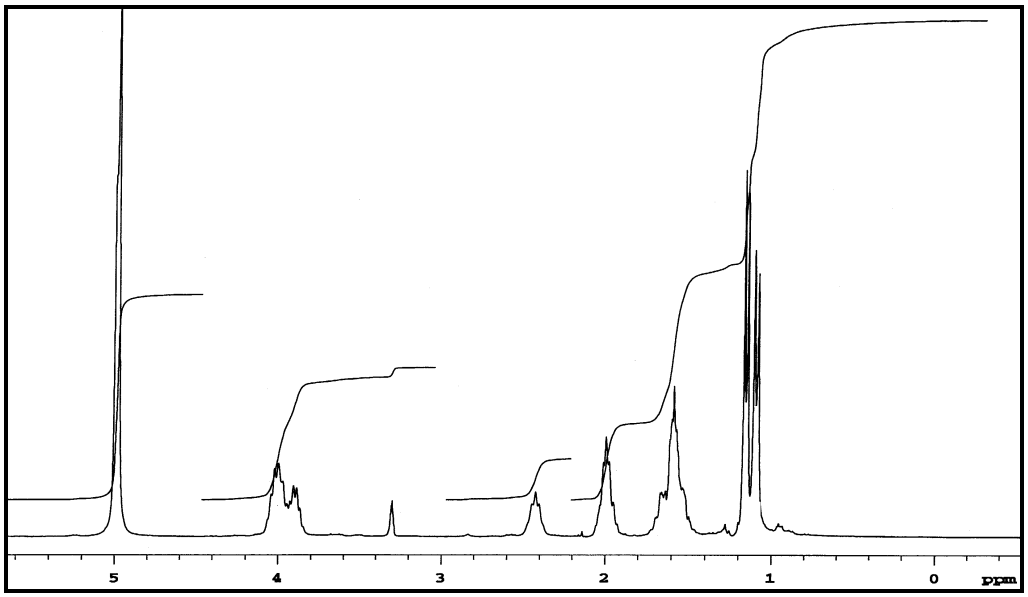


Figure 210: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of nonactic acid (**123**)

^{13}C NMR spectrum of **123** revealed 9 carbons, among them a quaternary carbon at δ 179.2 for an amide, acid or ester carbonyl. Three oxygenated methine carbons at δ 82.0, 77.9, 66.1 were observed. In addition one methine carbon was visible at δ 46.1. Two methylene groups at δ 32.2, 29.4 and two methyl groups at δ 24.1, 14.1 were also present.

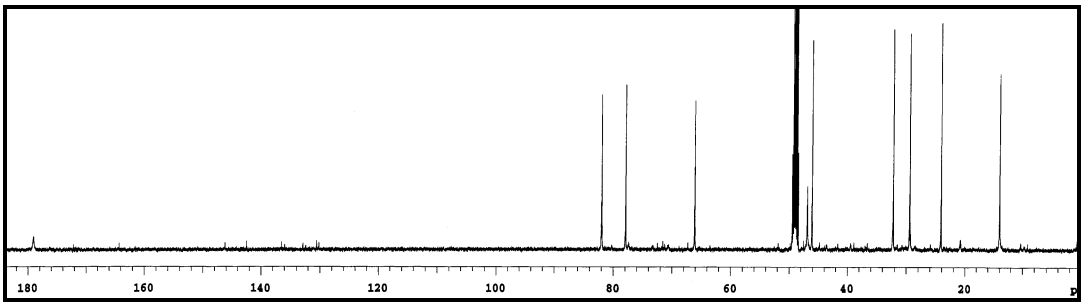
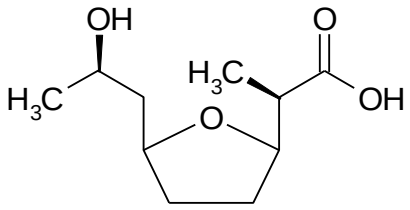


Figure 211: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of nonactic acid (**123**)

The ESI mass spectrum of compound **123** displayed *pseudo*molecular ion peaks at *m/z* 225 $[M+Na]^+$, 427 $[2M+Na]^+$ and at *m/z* 201 $[M-H]^-$ and 403 $[2M-H]^-$. A search in AntiBase supported by ^{1}H, ^{13}C NMR and mass spectroscopic data led to nonactic acid (**123**). The structure was confirmed by comparing with the literature[197] and authentic data. Compound **123** was synthesized by Bercedo *et al*.[198] and exhibited moderate inhibitory activity against 3α-hydroxysteroid dehydrogenase [199].



123

4.16.2 Homononactic acid

From the same subfraction, homononactic acid (**124**) was isolated as oily substance. The ^{1}H NMR spectrum revealed in the aliphatic region the same signals but showed a $[-CH_2CH_3]$ fragment instead of one methyl doublet.

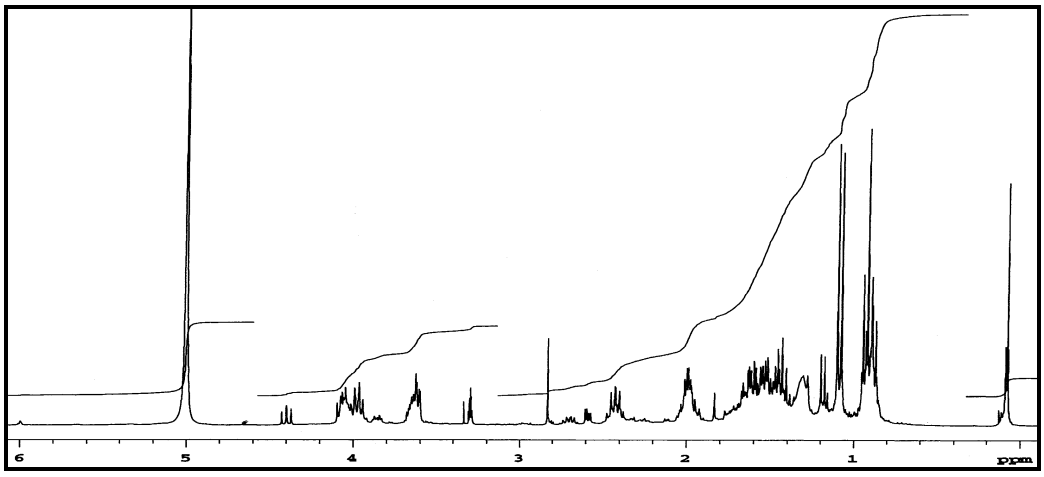


Figure 212: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of homononactic acid (**124**)

^{13}C NMR spectrum showed 11-carbon signals among carbon of acid, ester or amid, and methylene carbon at δ 31.4, which was not present in nonactic acid (**123).** The HRESIMS established the molecular formula as $C_{11}H_{20}O_4$.

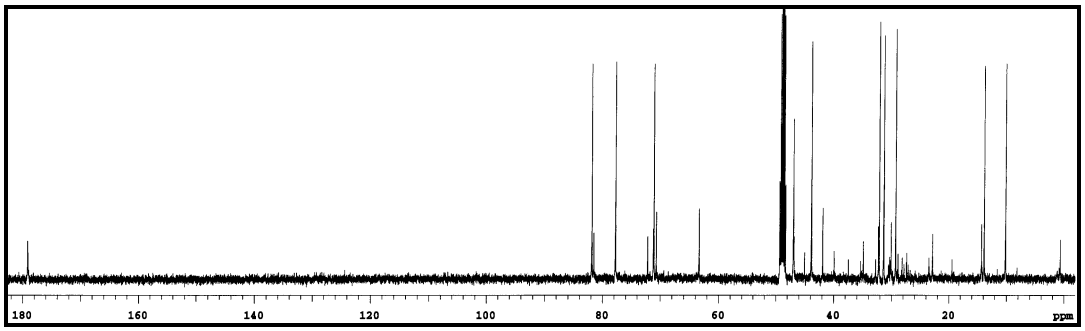
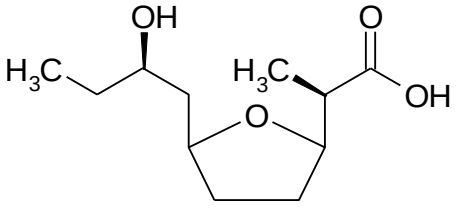


Figure 213: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of homononactic acid (**124**)

A search in AntiBase[77] with the spectroscopic data identified the compound as homononactic acid (**124**). The structure was confirmed by comparing with the literature. Homononactic acid (**124**) has been synthesized by Sharma *et al.*[200]



124

4.16.3 3-(3,3-Bisindolyl)propane-1,2-diol

The ^{1}H NMR spectrum of **125** revealed two overlapping 2H doublets at δ 7.54 and 7.28 and two overlapping 2H triplets at δ 7.01, 6.90, respectively. The pattern in the aromatic region suggested that there are two 1,2-disubstituted benzene rings. Additionally, there were two singlets at δ 7.29 and 7.13, giving evidence of two 3-substituted indole moieties. In the aliphatic region, diastereotopic methylene protons at δ 3.61 and 3.48 were visible, which indicated a neighbouring stereogenic centre and possibly an sp^2 carbon or heteroatom. Furthermore, two oxymethine protons were observed as doublet at δ 4.68 and multiplet at δ 4.48.

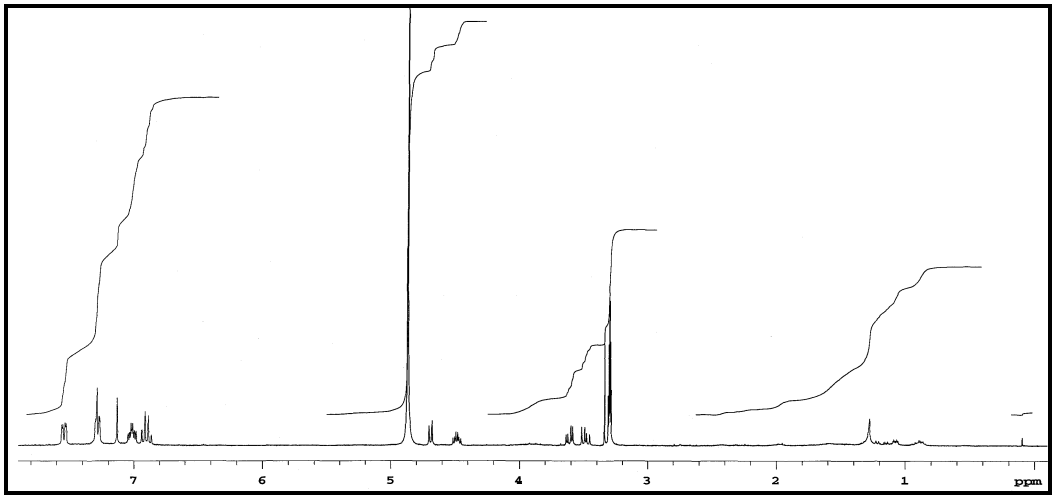
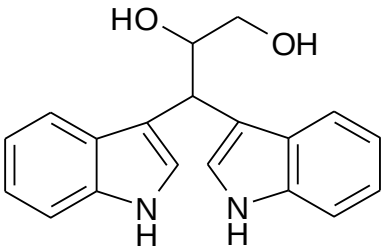


Figure 214: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 3-(3,3-bisindolyl)propane-1,2-diol (**125**)

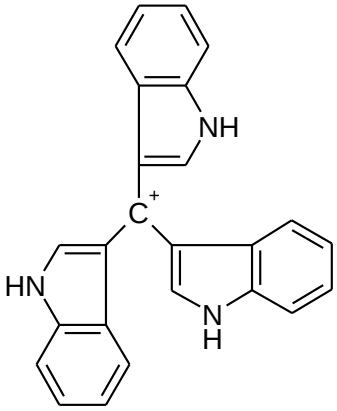
The ESI mass spectrum showed a *pseudo*molecular ion peak at *m/z* 329 $[M+Na]^+$, 635 $[2M+Na]^+$ and *pseudo*molecular ion peak at *m/z* 305 $[M-H]^-$ which fixed the molecular weight as 306 Dalton. Compound **125** was found to have a molecular formula of $C_{19}H_{18}N_2O_2$ by HRESI. A search in the Chemical Abstracts and AntiBase using ^{1}H and mass spectral data, led to 3-(3,3-bisindolyl)propane-1,2-diol (**125**). It was confirmed by comparing with literature values and authentic data.[201] 3-(3,3-bisindolyl)propane-1,2-diol (**125**) was isolated from the culture of an endophytic fungus EN-22 that was derived from the marine red alga *Polysiphonia urceolata*[202], and from the yeast of *Hansenula henricii*.[203] It was also isolated from the clavicipitaceous fungus *Balansia epichloe* and showed toxicity to fertilized leghorn eggs.[204]



125

4.16.4 Turbomycin A

Turbomycin A (**126**) was isolated as red oil with UV absorbing property. It turned to colourless on spraying with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of **126** revealed in the aromatic region five signals: a singlet at δ 8.24 (H-2), two doublets at δ 7.64, 6.90 and two triplets at δ 7.28, 7.01. The chemical shift and chemical pattern indicated an indole moiety. The ESIMS showed a *pseudo*molecular ion at *m/z* 358 $[M-H]^-$ and *m/z* 360 $[M]^+$ corresponding to a molecular weight of 359 Dalton. The odd mass number was an indication for an odd number of nitrogen atoms in the molecule. HRESIMS established the molecular formula as $C_{25}H_{17}N_3$ and confirmed the presence of three nitrogen atoms in this compound. A search in the Chemical Abstracts and our own database using ^{1}H and mass spectroscopic data led to turbomycin A (**126**). Turbomycin A (**126**) and B were extracted from P57G4 as a red and an orange pigment respectively. Both exhibited antibiotic activities against gram-negative and positive bacteriea. Turbomycin A (**126**) and turbomycin B are formed by interaction of indole with indole-3-carbaldehyde.[205]



126

4.17 *Trichoderma* sp. HMM2

The crude extract of the *Trichoderma* sp. HMM2 showed strong biological activity against the test microorganisms, see Table 26. TLC analysis exhibited different coloured reactions with anisaldehyde/sulphuric acid.

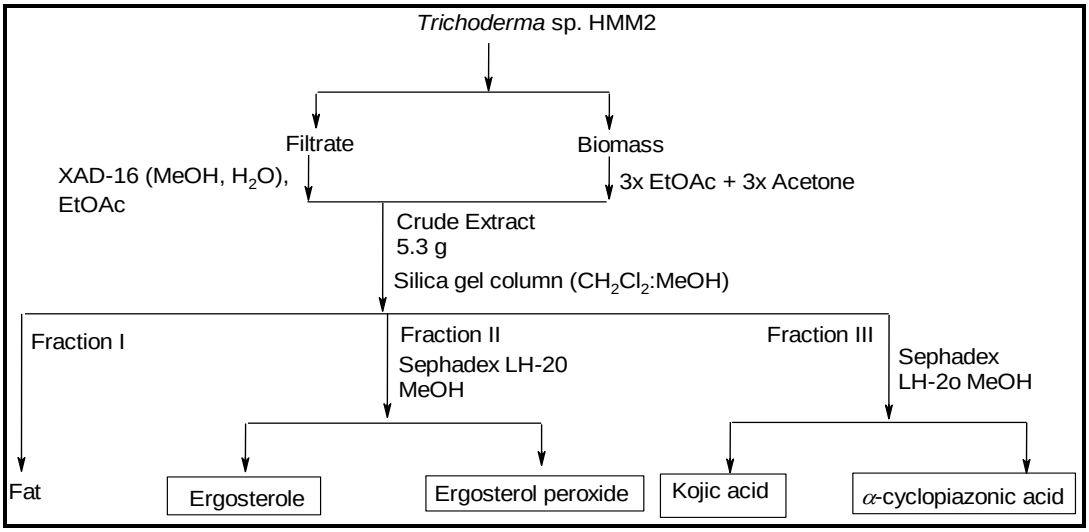


Figure 215: Work-up scheme of *Trichoderma* sp. HMM2

4.17.1 Kojic acid

Kojic acid (**127**) was isolated as white solid from fraction III. On TLC it gave a UV absorbing band, which turned to blue with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum revealed two broad signals at δ 8.99 and δ 5.69 for two acidic exchangeable protons, a 1H singlet at δ 7.97 supposed to be attached to a heteroatom and another singlet at δ 6.34 in the aromatic region. In the aliphatic region a methylene group attached to oxygen was seen at δ 4.28.

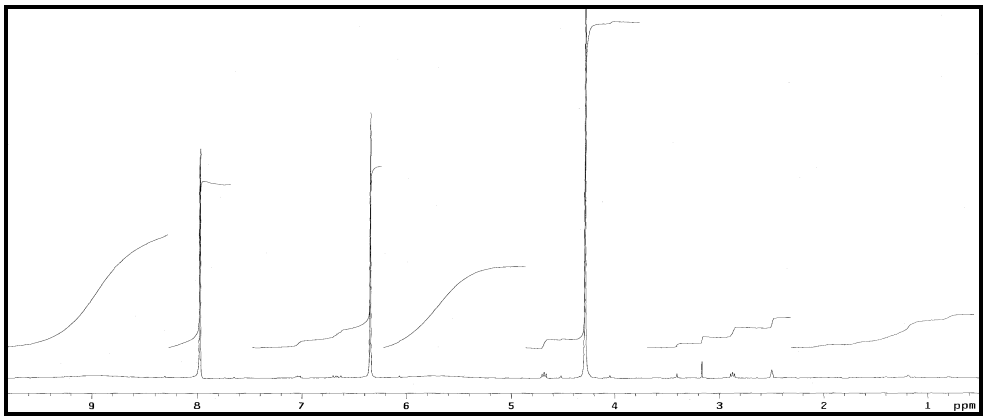
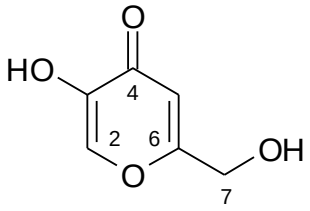


Figure 216: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of kojic acid (**127**)

^{13}C NMR spectrum of **127** exhibited a carbonyl signal at δ 174.2 for acid, ester or amid, in addition to a quaternary carbon attached to a heteroatom at δ 168.3. Furthermore, two carbons at δ 153.0 and 138.9, which represented a double bond fragment in conjugation with a carbonyl group, as well as one aromatic signal at δ 110.0 and methylene carbon at δ 59.7 were observed.



127

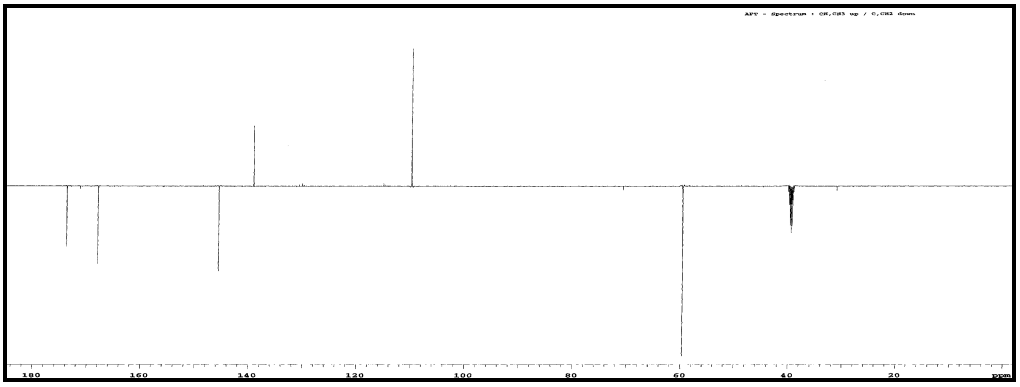


Figure 217: ^{13}C/APT NMR spectrum (DMSO-d_6, 125 MHz) for kojic acid (**127**)

Kojic acid (**127**) is known since a long time. Recently, it was again isolated from the broth of the fungus *Paecilomyces lilacinus*, which was derived from a marine sponge *Petrosia* sp.[206], found in a toxigenic strain of *Aspergillus parasiticus* and a non-toxigenic strain of *Aspergillus flavus*.[207]

4.17.2 Ergosterol

Ergosterol (**128**) was isolated as a colourless solid from a middle polar fraction and visualised by the violet colour with anisaldehyde/sulphuric acid. The ^{1}H NMR spectrum of **128** exhibited in the olefinic region two protons as doublets of doublets at δ 5.48 and 5.34, and two proton signals overlapping at δ 5.24-5.26. In the aliphatic region a multiplet at δ 3.40, methylene multiplets at δ 2.35, 2.17, a methyl singlet at δ 0.62, and four methyl doublets at δ 0.82, 0.85, 0.91, 1.03 were observed. One me-

thyl singlet at δ 0.94 and many methylene protons overlapping at δ 2.00-1.17 were seen.

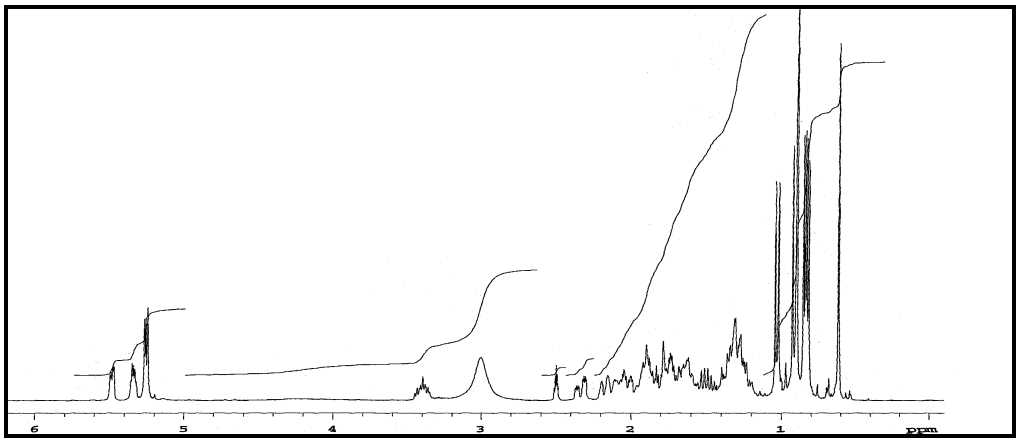
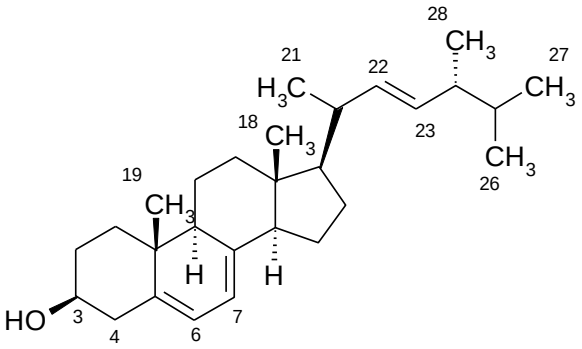


Figure 218: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of ergosterol (**128**)

The ESI mass spectrum of **128** displayed *pseudo*molecular ion peaks at *m/z* 419 $[M+Na]^+$ and 815 $[2M+Na]^+$, respectively. According to the spectral data and a search in AntiBase this compound was identified as ergosterol (**128**); it was confirmed with literature data[208, 209] and authentic spectra.



128

Ergosterol (**128**) was isolated from mycelial growth of strain 133 (DMI-sensitive) and strain 179 (DMI resistant).[209] Ergosterol is a general fungal metabolite

4.17.3 Ergosterol peroxide

Ergosterol peroxide (**129**) was isolated as a colourless UV absorbing solid, which turned to violet on spraying with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum of **129** exhibited in the olefinic region a 2H doublet at δ 6.52 (J = 8.5), 6.24 (J = 8.5), two protons overlapping at δ 5.20 (J = 15.5, J = 7.5), and in the

aliphatic region an oxymethine multiplet at δ 3.76 and a methylene triplet at δ 2.21 (J = 15.0, J = 7.4). Many methylene groups overlapped at 2.00-1.00. Additionally, four methyl groups were observed as doublets at δ 0.78, 0.86, 0.95, and 0.99.

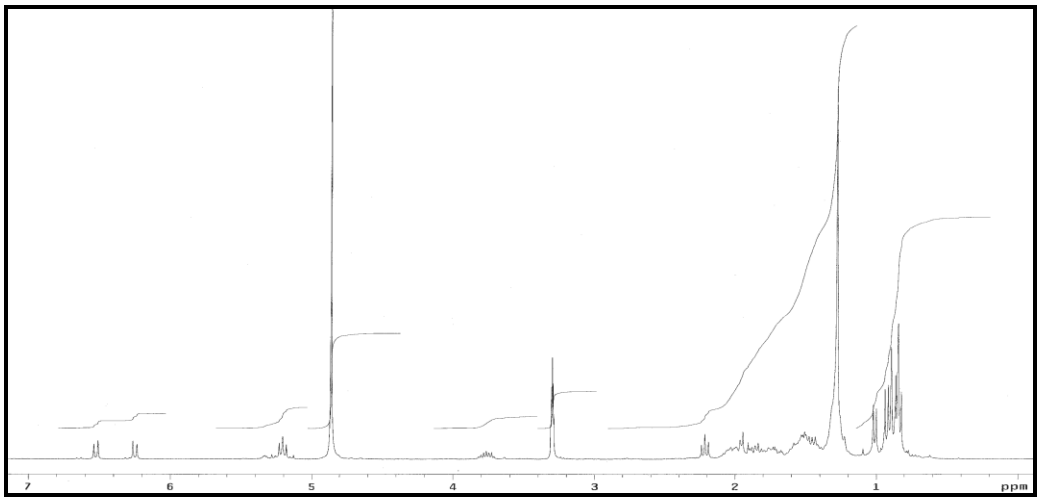
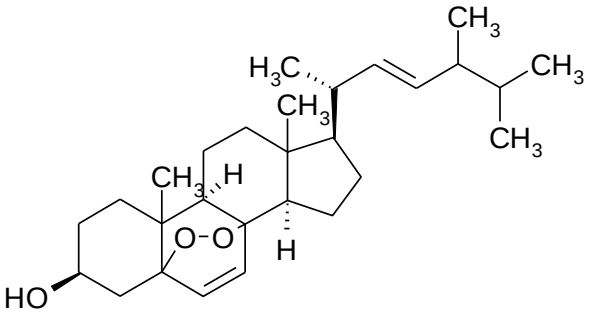


Figure 219: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of ergosterol peroxide (**129**)

The ESI mass spectrum of compound **129** displayed signals at *m/z* 451 $[M+Na]^+$, 879.7 $[2M+Na]^+$, 1308.0 $[3M+Na]^+$. According to this spectroscopic information and a search in AntiBase, the compound was identified as ergosterol peroxide (**129**). The structure was confirmed by literature and authentic spectra.[208]



129

Sterols are involved in the control of membrane related metabolic processes. They are of high importance for the physiology and biochemistry of all eukaryonts.[210]

4.17.4 *α*-Cyclopiazonic acid

The ESI mass spectrum of compound **130** displayed signals at *m/z* 359 $[M+Na]^+$, 695 $[2M+Na]^+$ in positive mode. In negative mode it displayed signals at *m/z* 335 $[M-H]^-$, and 693 $[2M+Na-2H]^-$. HRESIMS established the molecular formula $C_{20}H_{20}N_2O_3$ with twelve double bond equivalents. In the ^1H NMR spectrum, four proton signals were observed in the aromatic region: three adjacent protons of a tri-substituted benzene ring at δ 7.13, 7.05 and 6.98 as ABC system, in addition to a singlet at δ 7.10 for another ring. In the upfield region, three methine protons at δ 3.97 attached to heteroatom were observed. Further methine multiplets were observed at δ 3.58 and at δ 2.50, a methylene at δ 3.00, in addition to a methyl singlet at δ 2.40 bound to sp^2 carbon, and two further methyl groups at δ 1.60 and 1.55 were visible in the spectrum.

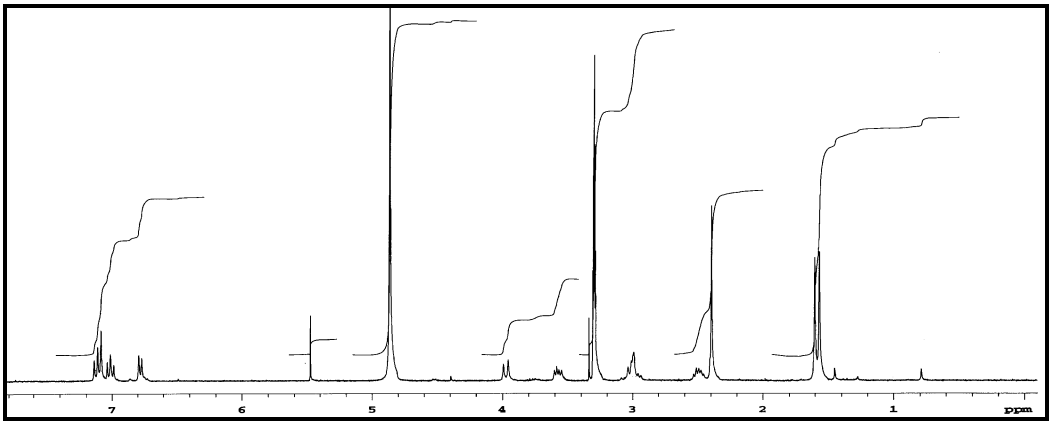


Figure 220: ^1H NMR spectrum (CD_3OD, 300 MHz) of *α*-cyclopiazonic acid (**130**)

The ^{13}C NMR spectrum of **130** showed 17 carbon signals including six quaternary carbons and four olefinic methine carbons between δ 123-109. In addition three methine carbons at δ 72.2, 55.0 and 37.8, one methylene at δ 27.7 and three methyls at δ 36.8, 26.4 and 25.2 were exhibited.

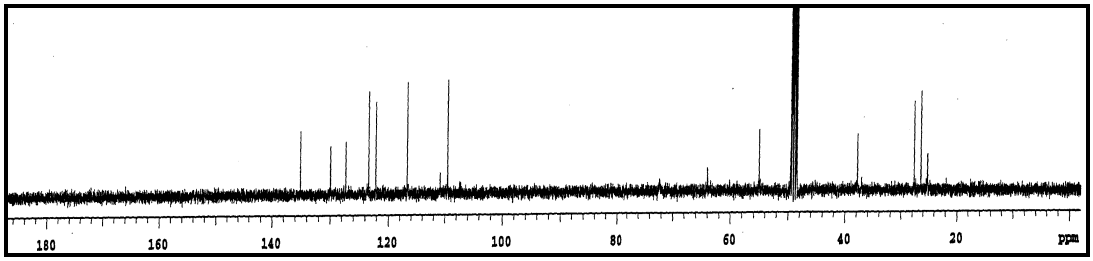
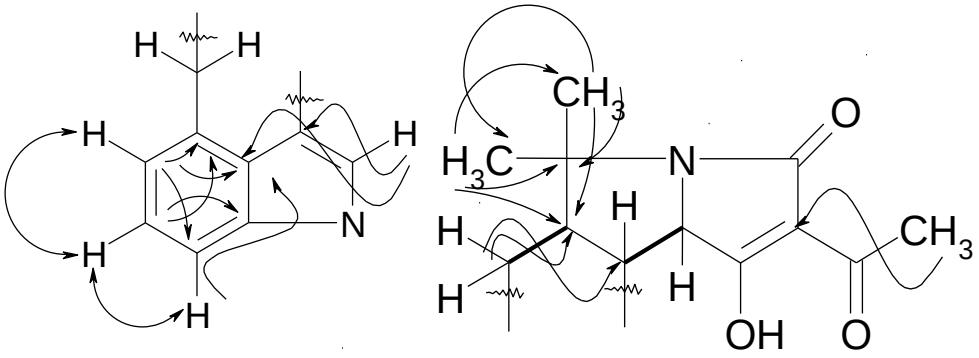


Figure 221: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of *α*-cyclopiazonic acid (**130**)

The HMBC spectrum showed many correlations. The proton at δ 6.98 showed ^{3}J correlation with quaternary carbons at δ 109.6 and 127.3 confirming the ABC system of a benzene ring, as well as with methylene group at δ 3.00. Moreover, the proton at δ 7.10 showed strong correlation with quaternary carbons at δ 110.9 and 127.3 (Fragment **A**). In addition two methyl singlets at δ 1.60 and 1.55 exhibited HMBC correlations with the quaternary carbon at δ 64.0 and a methine group at δ 55.0. The former methylene protons exhibited strong correlation with the methine carbons at δ 55.0 and 37.8. Finally, the methyl group at δ 2.40 correlated with the quaternary carbon at δ 106.2 (Fragment **B**).



Fragment **A** Fragment **B**

Figure 222: HMBC correlations of substructures of α-cyclopiazonic acid (**130**)

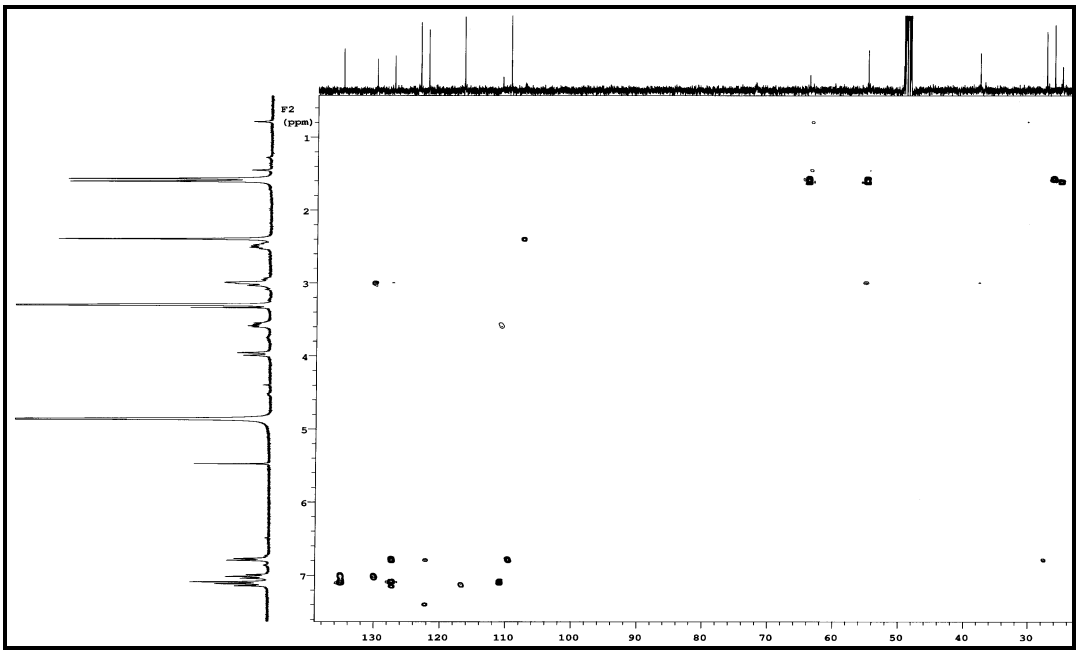
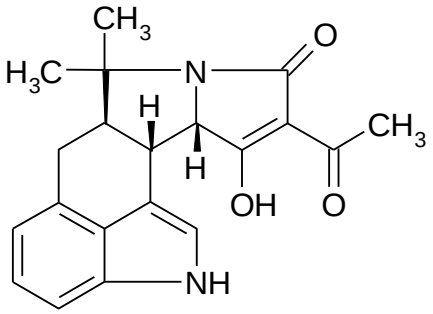
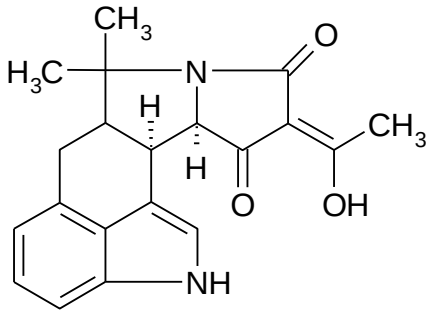


Figure 223: HMBC spectrum (CD_3OD, 500 MHz) of α-cyclopiazonic acid (**130**)

A search in AntiBase supported by ^{1}H, ^{13}C NMR, 2D and MS spectroscopic data led to the alkaloid, *α*-cyclopiazonic acid (**130**), which was further confirmed by the literature data[211]. *α*-Cyclopiazonic acid (**130**) was found in peanuts[212], and was isolated with its isomer iso-*α*-cyclopiazonic acid (**131**) from the marine-derived fungus *Aspergillus flavus* C-F-3.[213] It was also isolated from a marine-derived fungus *Aspergillus tamarii* and the structure was determined by crystal X-ray diffraction analysis.[214] The fungi *Aspergillus flavus* and *Aspergillus oryzae* also produced this compound; it shows moderate cytotoxicity.[211]



130



131

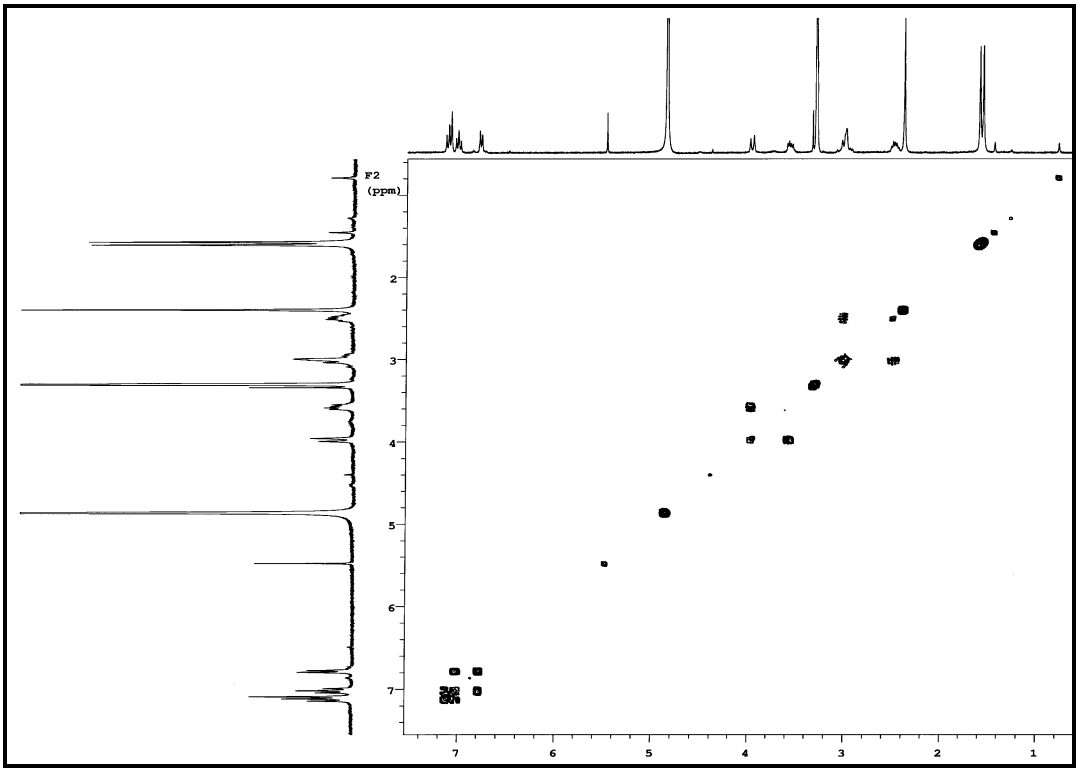


Figure 224: $^{1}H,^{1}H$ COSY spectrum (CD_3OD, 500 MHz) of *α*-cyclopiazonic acid (**130**)

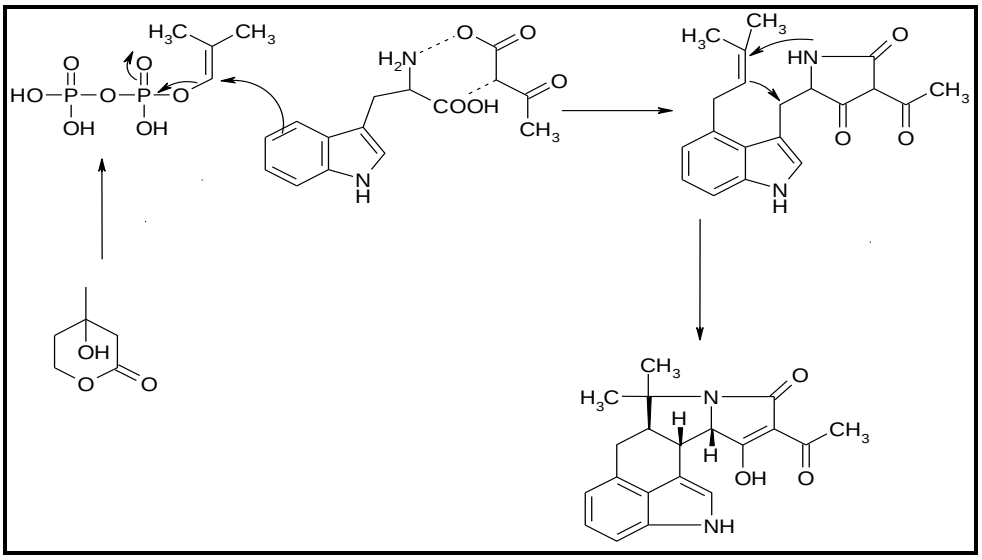


Figure 225: Biosynthetic pathway of α-cyclopiazonic acid (**130**).[215]

4.18 *Aspergillus oryzae* sp.

The crude extract of the *Aspergillus oryzae* sp. showed strong biological activity against the tested microorganisms, see Table **27**, and the TLC analysis exhibited different colour reactions with anisaldehyde/sulphuric acid and heating.

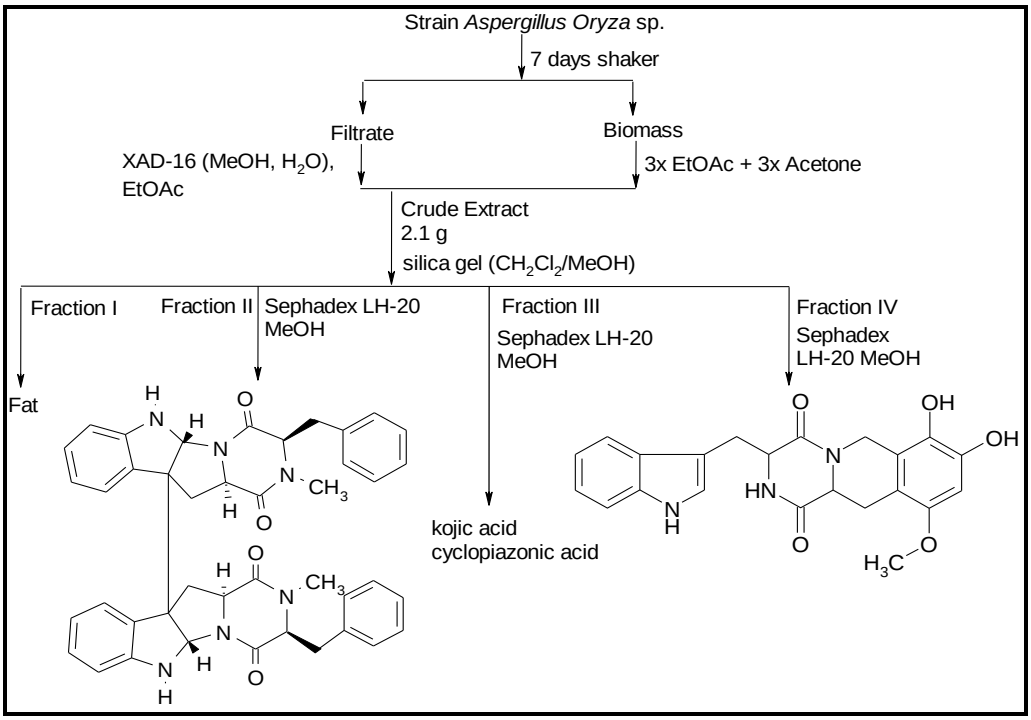


Figure 226: Work-up scheme of *Aspergillus oryzae* sp.

4.18.1 7,9-Dihydroxy-3-(1H-indol-3-ylmethyl)-8-methoxy-2,3,11,11a-tetrahydro-6H-pyrazino[1,2-b]isoquinoline-1,4-dione

The molecular weight of **134** was deduced from the ESI mass spectrum, which showed *pseudo*molecular ions at *m/z* 406 $[M-H]^-$, 813 $[2M-H]^-$, and at *m/z* 430 $[M+Na]^+$ and 837 $[2M+Na]^+$, corresponding to a molecular weight of 407 Dalton. The odd mass number is an indication of an odd number of nitrogen atoms in the molecular formula. HRESIMS established the empirical molecular formula as $C_{22}H_{21}N_3O_5$. The ^{1}H NMR spectrum of **134** showed four acidic exchangeable protons at δ 10.83, 9.04, 8.81 and 8.33 for two secondary amine and amide and two hydroxyl protons. In addition there were five aromatic protons: two appeared as doublets at δ 7.50 (J = 7.8), 7.24 (J = 8.0) and further two protons appeared as triplets at δ 6.18 (J = 7.6) and 6.91 (J = 7.6), which gave the pattern of an 1,2-disubstituted benzene ring. A 1H singlet overlapped at δ 6.93, which is characteristic for an indole moiety. An olefinic singlet was seen at δ 5.85. In the aliphatic region two methylene groups were visible: one as ABX system at δ 2.33 and 0.96 connected with an sp^2 carbon and another methylene group at δ 4.96 and 3.71, connected to a heteroatom and/or sp^2 carbon. Moreover two methine protons at δ 4.33 and 3.78 attached to heteroatom, and finally one methoxy at δ 3.63 was observed.

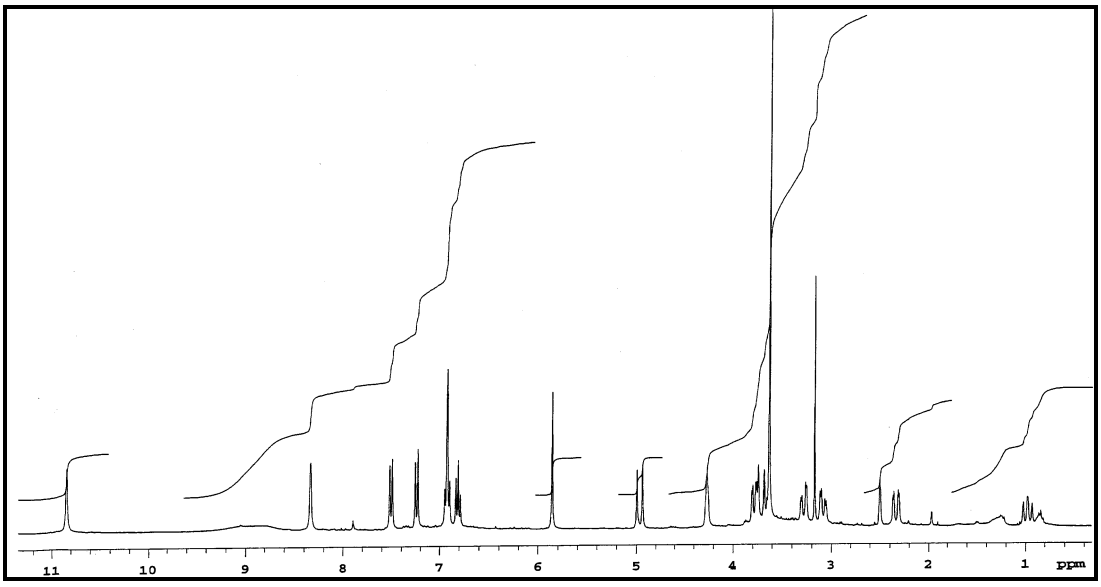


Figure 227: ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) of compound **134**

^{13}C NMR and HMQC spectra of **134** revealed 22 carbon signals including two carbonyl carbons at δ 165.5 and 164.2 for amides or esters, and 14 sp^2 carbons: six

methines and eight quaternary carbons. A methoxy carbon at δ 59.9 was observed, in addition to two methine signals δ 55.7 and 54.6. Finally two methylene carbons at δ 32.2 and 29.9 were observed.

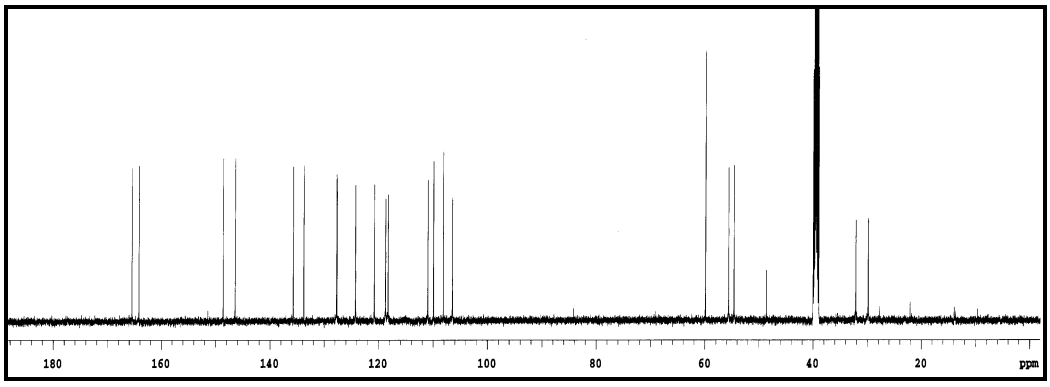


Figure 228: ^{13}C NMR spectrum (DMSO-d_6, 125 MHz) of compound (**134**)

The ^{1}H,^{1}H COSY spectrum of **134** displayed ^{3}J correlations from the doublet at δ 7.50 (H-4) to a triplet at δ 6.18 (H-5). The latter showed strong coupling with a triplet at δ 6.91 (H-6), and further a strong correlation with the doublet at δ 7.24. The connectivity was also confirmed by further HMBC correlations from H-4 to δ 120.8 (C-6), δ 127.7 (C-3a), δ 108.1 (C-3) and δ 135.7 (C-7a), which suggested a 1,2-disubstituted benzene ring. In addition strong correlations in the ^{1}H,^{1}H COSY spectrum from the acidic proton at δ 10.85 to the singlet at δ 6.93 (H-2), which itself showed HMBC correlations with δ 127.7 (C-3a), δ 108.1 (C-3), and δ 135.7 (C-7a) confirmed the indole moiety (fragment **A**). The methylene group CH_2-8 showed strong correlations to the methine proton at δ 4.33 (CH-9); the latter showed a strong HMBC correlations to two amide carbonyls at δ_c 164.2 and 165.5 and gave a diketopiperazine ring (fragment **B**), confirmed by strong correlations from the methine proton at δ 3.78 to the amide carbonyls. Methylene protons of CH_2-8 and the methine at δ 4.33 (CH-9) exhibited strong ^{3}J correlations to the methine proton at δ 6.93 and quaternary carbon at δ 108.1 (C_q-3), respectively, which suggested a connection between fragment **A** and fragment **B**.

The HMBC spectrum of **134** exhibited strong correlations from the olefinic singlet at δ 5.85 to oxy-sp^2 carbons at δ 146.4 and 148.6 and the quaternary carbon at δ 133.8, which displayed strong correlations with the methoxy singlet at δ 59.9. In addition, the methylene protons at δ 4.96, 3.71 displayed strong correlation to both qua-

ternary carbons at δ 127.6, 109.9 and to oxygenated quaternary carbon at δ 146.4 and carbonyl at δ 164.2 and to methine carbon at δ 3.78. Additionally there was a strong correlation between the amide carbonyl at δ 164.2 and the methine proton at δ 3.78, which displayed strong ^{1}H,^{1}H COSY correlations with the methylene protons at δ 2.33 and 0.96. Additional correlations from methine proton δ 3.78 to the other amide carbonyl at δ 165.5 led to fragment **C**. All these correlations confirmed the connection of fragment **B** with fragment **C**. the substructure confirmed as tetrahydroisoquinoline skeleton with one methoxy and two hydroxy groups.

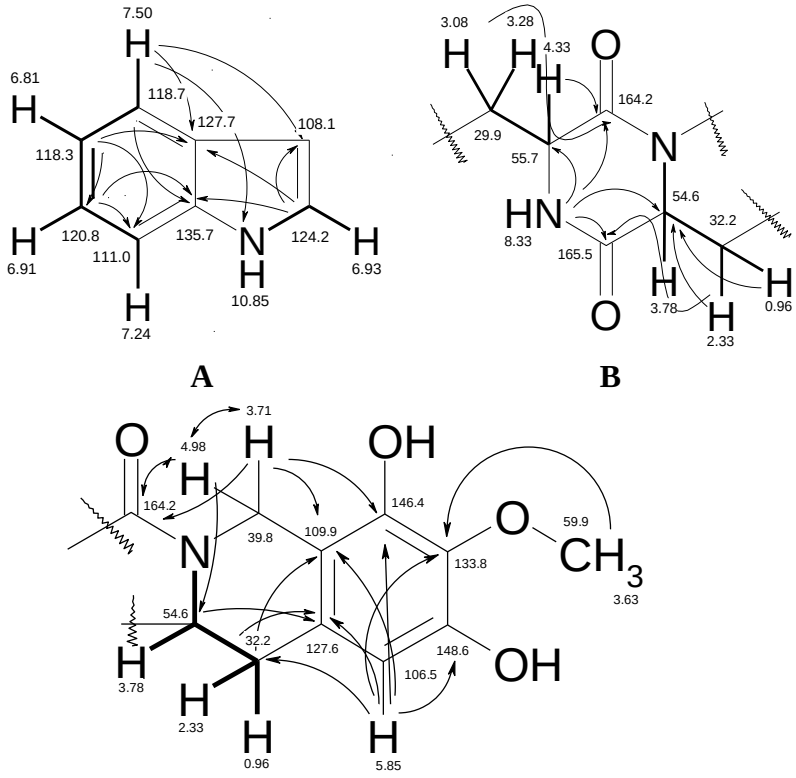


Figure 229: Substructures and selected ^{1}H,^{1}H COSY (▬) and HMBC (→) correlations of compound **134**

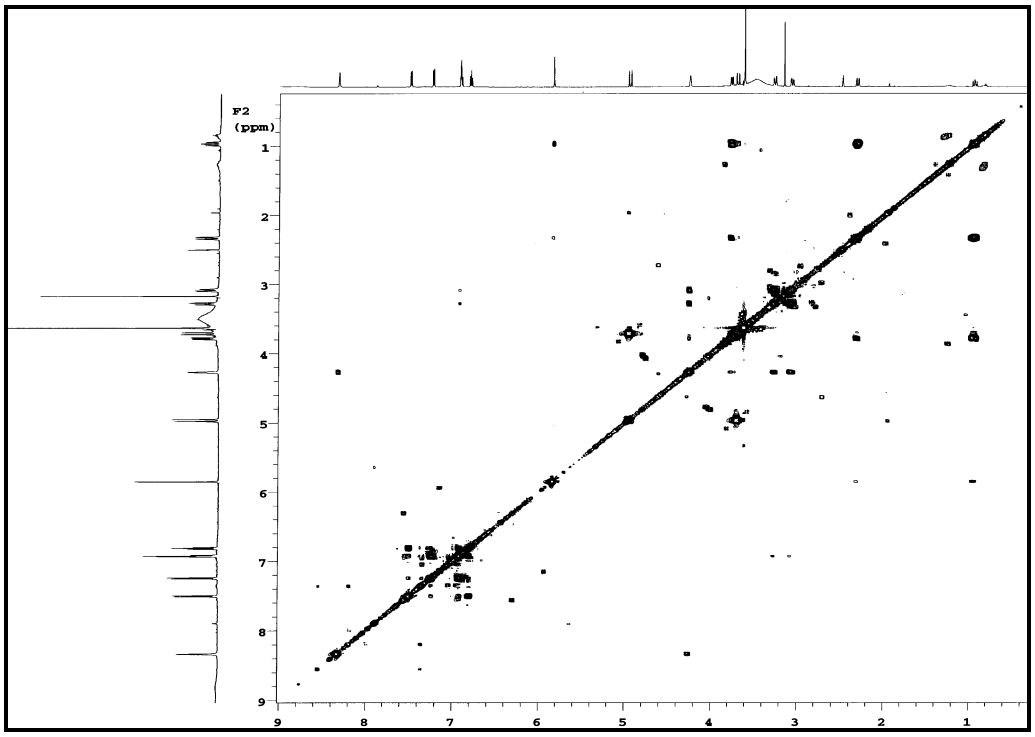
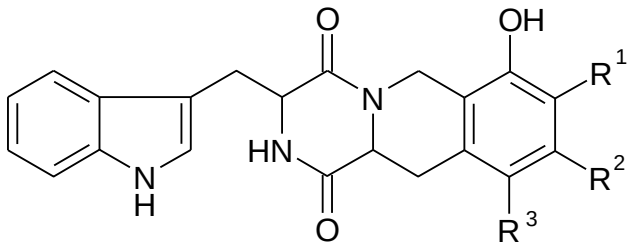


Figure 230: ^{1}H,^{1}H COSY spectrum (DMSO-d_6, 500 MHz) of compound **134**

According to calculation using COCON[216] and considering the strong ^{3}J correlation which led to two possible structures **133** and **134**, and the ^{4}J correlations, **132** and **134** were obtained. As the singlet at δ 5.85 showed NOE-correlations with methylene at δ 2.33, 0.96, structure **133** was ruled out. So the structure was determined as **134**.



	R^1	R^2	R^3
132	OCH3	H	OH
133	OH	OCH_3	H
134	OCH_3	OH	H

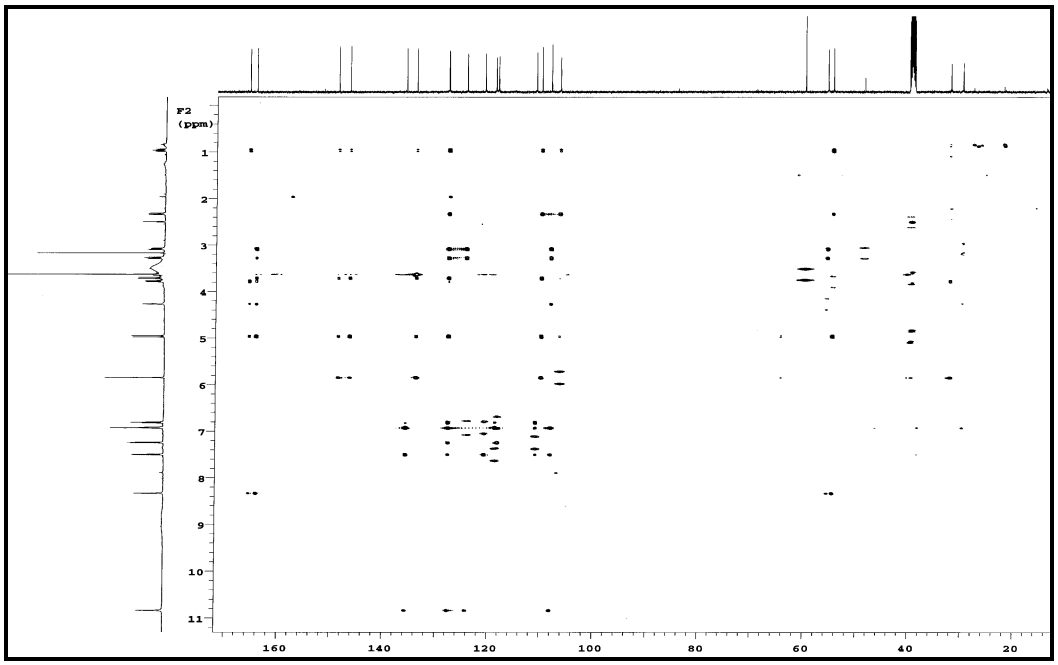
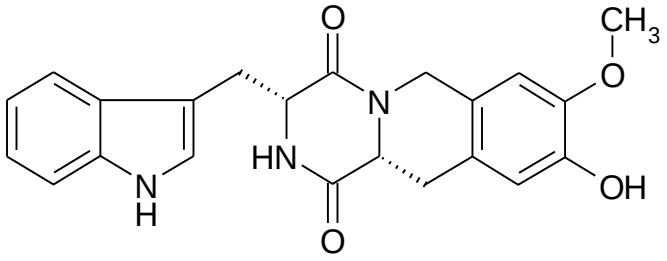


Figure 231: HMBC spectrum (DMSO-d_6, 500 MHz) of compound **134**

Compound **134** is closely related to the dipeptidyl peptidase IV (DPIV) inhibitors TMC-2A, B and C which were obtained from the fermentation broth of *Aspergillus oryzae* A374. TMC-2A, B and C inhibited rat kidney DPIV with IC50 value of 8.1 μM, 17 μM, and 20 μM, respectively. The configuration of **134** was deduced on the basis of NOESY experiments: The proton signals of both chiral centres H-2 (4.33) and H-2' (3.78) did not show a correlation to each other indicating that the *trans* configuration is more plausible; the absolute configuration was not determined. A compound closely related to **134** is **135**, which was isolated from an algicolous *Aspergillus flavus*[217]; it showed weak cytotoxicity against HL-60 cell lines with an IC_{50} value of 36.5 μg/ml. Also the high negative optical rotation (-200°) as well as to the optical rotation for **135** (-228.7°) are confirming their similarity.

Table 17: ^{13}C (125 MHz) and ^{1}H NMR (300 MHz) data of **134** (DMSO-d_6; shifts as δ values, J in [Hz]).

Position	δ_C	δ_H	HMBC
1	164.2	-	-
2	55.7	4.26 (s)	1, 1', 4, 5
3	29.9	3.28 (ddd, 14.2, 4.2, 3.2), 3.08 (ddd, 14.2, 4.2, 3.9)	1,2,4,5, 10a
4	108.1	-	-
5	124.2	6.92 (s)	3, 4, 7a, 10a
6-NH	-	10.85 (s)	5, 4, 7a, 10a
7a	135.7	-	-
7	111.0	7.24 (d, 8.0)	10a, 9, 8
8	120.8	6.92 (t, 7.6)	7a, 7, 9, 10
9	118.3	6.81 (t, 7.6)	10a, 10, 8, 7
10	118.7	7.50 (d, 7.8)	7a, 10a, 9, 8, 4
10a	127.7	-	-
1'	165.5	-	-
2'	54.6	3.78 (ddd, 12.2, 3.2, 3.7)	1, 1', 3', 4'
3'	32.2	2.33 (dd, 15.8, 3.2), 0.96 (t, 13.9)	1', 2', 4', 5', 9'
4'	127.6	-	-
5'	106.5	5.85 (s)	3', 6', 7', 8', 9', 10'
6'	148,6	-	-
7'	133.8	-	-
7'-OCH_3	59.9	3.63 (s)	7'
8'	146.4	-	-
9'	109.9	-	-
10'	39.8	4.96 (d, 17.1), 3.71 (d, 17.1)	1, 1', 2', 9', 4', 8', 5'
OH	-	9.04 (s br)	
OH	-	8.81 (s br)	
2-NH	-	8.34 (s)	



135

4.18.2 Ditryptophenaline

Ditryptophenaline (**136**) was isolated as a colourless solid from a UV absorbing band, which changed to yellow by spraying with anisaldehyde reagent and heating. The ^{1}H NMR spectrum of **136** exhibited in the aromatic region five proton signals as an indication of a phenyl ring. Three of them were two multiplets overlapping at δ 7.51 and one was a triplet at δ 7.46, while further two protons appeared in the same position at δ 7.10. In addition, four protons, two as triplets at δ 7.03 and 6.66 and the other two as doublets of doublets at δ 6.93 and 6.51 were observed, which suggested a 1,2-disubstituted benzene ring. In the aliphatic region one broad singlet of an acidic exchangeable proton at δ 4.75, as well as three methine protons at δ 4.78, 4.21 and 3.61 attached to heteroatoms were observed. In addition, two methylene multiplets appeared at δ 3.48, 3.20 and at δ 1.98, 1.53 and were attached to an sp^2 carbon or a chiral centre, respectively. Finally a methyl singlet was found at δ 2.98.

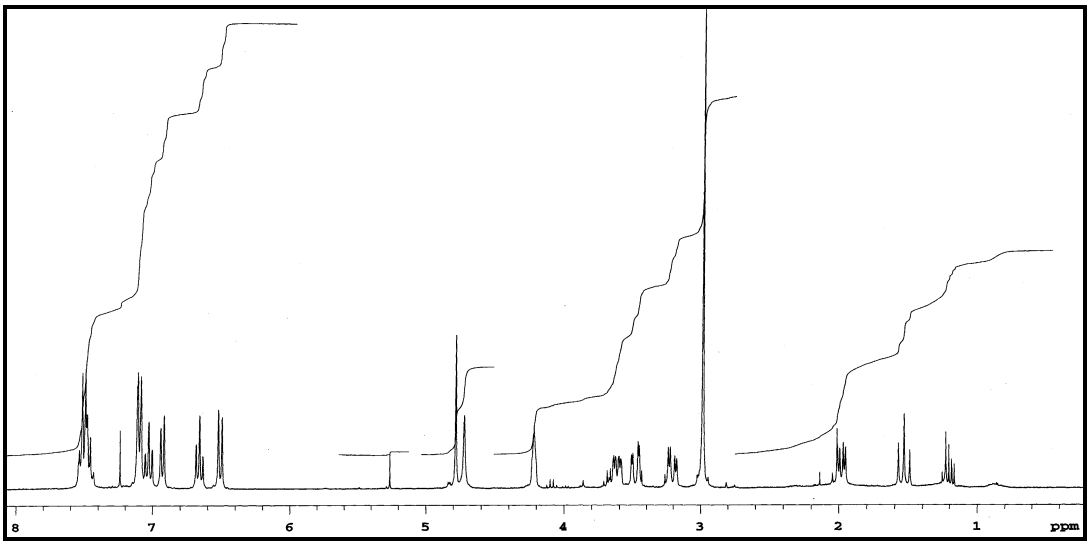


Figure 232: ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of ditryptophenaline (**136**)

The ^{13}C NMR and HMQC spectra of **136** displayed 21 carbon signals including two amide carbonyls at δ 165.3 and 163.9, 11 quaternary sp^2 carbons and an aromatic methine proton. The aliphatic region displayed a quaternary carbon at δ 58.5, three methine carbons at δ 78.6, 63.0 and 58.9 attached to heteroatoms, and two methylene carbons at δ 36.1 and 35.9. Finally a methyl group at δ 32.5 was seen.

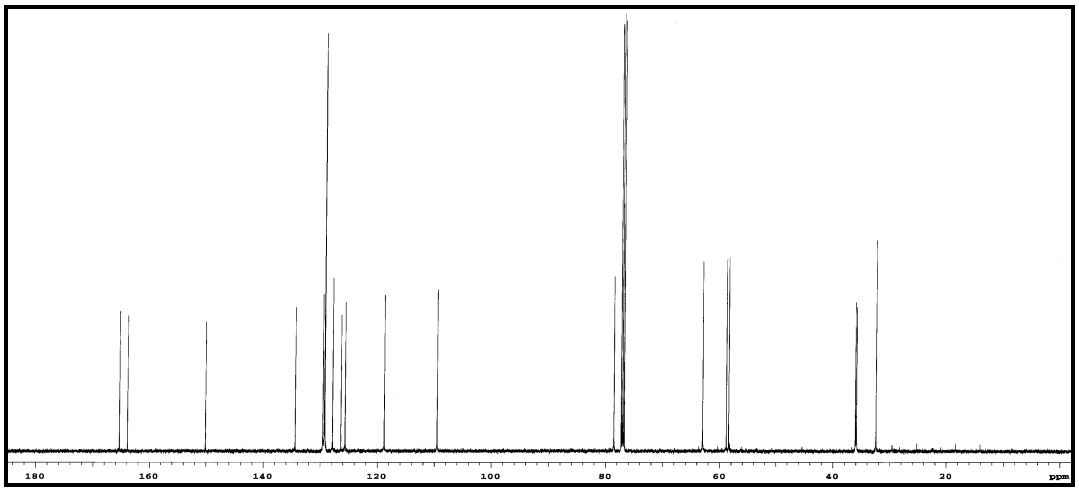


Figure 233: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of ditryptophenaline (**136**)

The ^{1}H,^{1}H COSY spectrum exhibited strong correlation from the triplet at δ 7.03 to a doublet at δ 6.51 and a triplet at δ 6.66. This proton exhibited strong correlation to a doublet at δ 6.93, which showed a long-range coupling in HMBC spectrum of **136** to aromatic carbons at δ 126.4 and 150.1, suggesting the connection with a heteroatom. The latter quaternary carbon showed a strong ^{3}J coupling from the methine proton at δ 4.78 suggesting the linkage via nitrogen. In addition there were strong correlations from the latter proton at δ 4.78 to two quaternary carbons at δ 126.4 and 58.5. Also the methine singlet at δ 4.78 exhibited strong correlation to the methylene carbon at δ 35.9 that correlated to another methine proton at δ 3.61 in the COSY spectrum (fragment **A**). The methine carbon at δ 58.9 (δ_H 3.61) exhibited strong correlations in the HMBC spectrum to two amide carbonyls at δ 163.9 and 165.3. Furthermore the second carbonyl at δ 165.3 showed strong correlation with the methyl singlet at δ 32.5 and with the methine doublet at δ 4.21. The latter showed ^{3}J coupling to another carbonyl of amide at δ 163.9 to confirm the piprazinedione moiety (Fragment **B**), which was connected to Fragment **A**. The ^{1}H,^{1}H COSY spectrum displayed strong correlation from two protons at δ 7.51 to a triplet at δ 7.46. Additionally the doublet at δ 7.10 displayed also strong correlation to the proton at 7.51 to suggest a phenyl group. This was confirmed by HMBC correlations of **136**. Strong correlations from the methine proton at δ 4.21 to the quaternary carbon at δ 134.4 were observed. The latter showed ^{2}J correlations also from methylene protons at δ 3.48 and 3.20 (fragment **C**) to connect with fragment **B** to give fragment **D**.

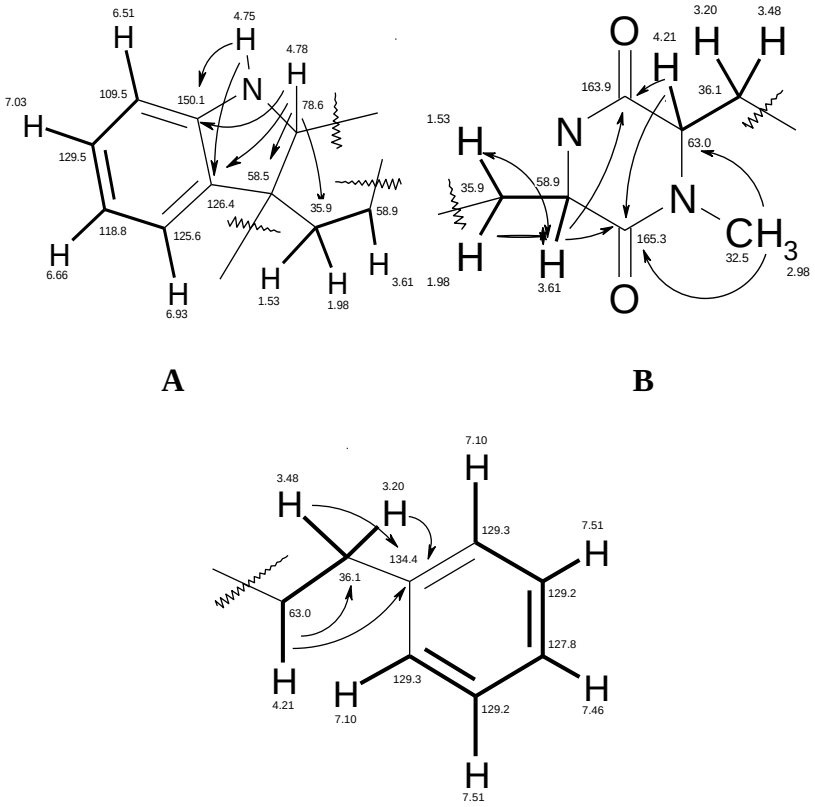


Figure 234: $^{1}H,^{1}H$ COSY (▬) and HMBC (→) correlation of ditryptophenaline (**136**)

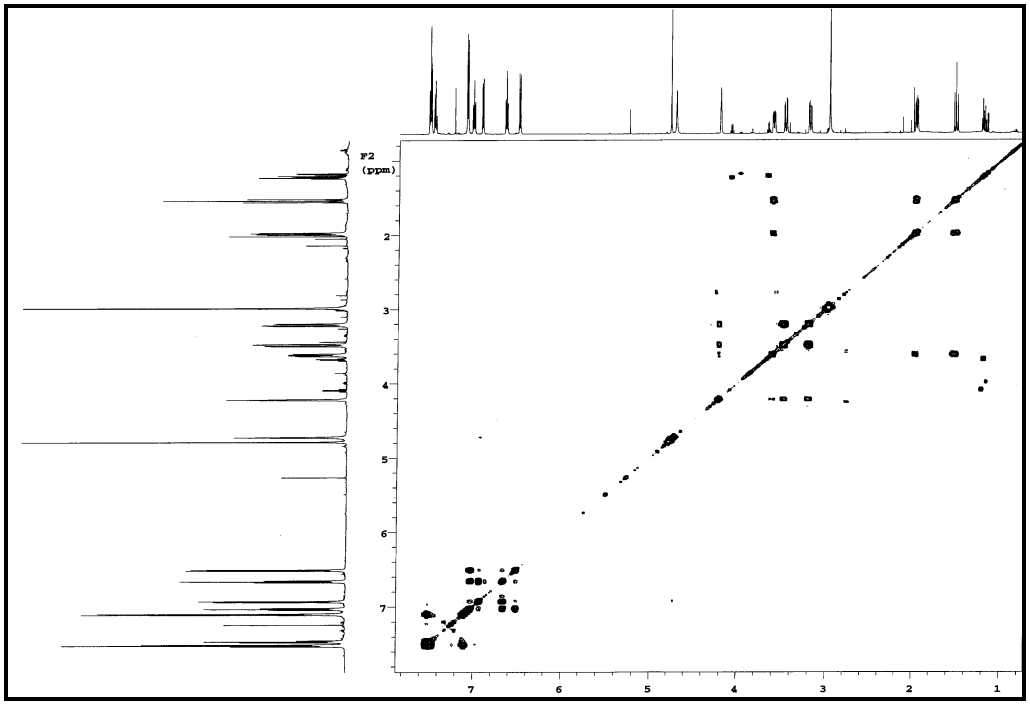
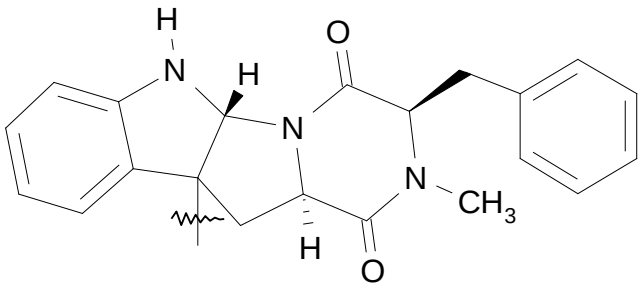


Figure 235: $^{1}H,^{1}H$ COSY spectrum ($CDCl_3$, 600 MHz) of ditryptophenaline (**136**)



D

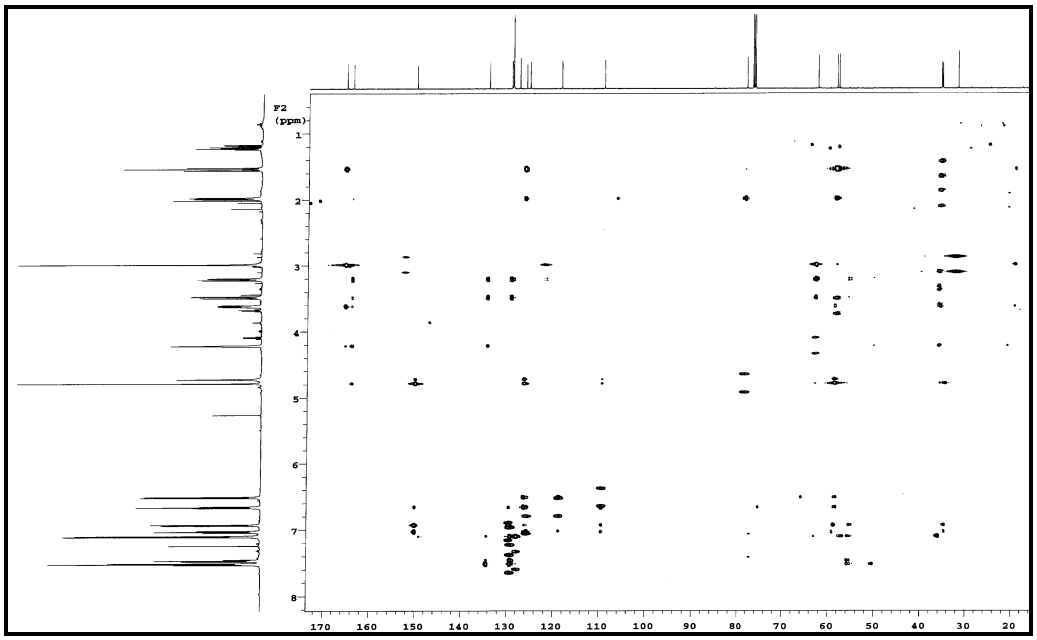
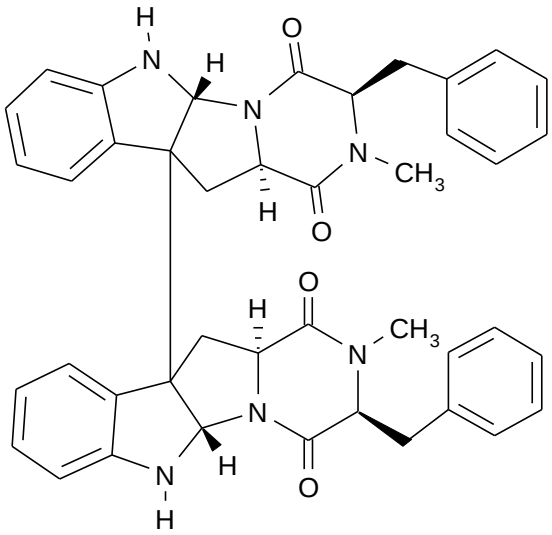


Figure 236: HMBC spectrum ($CDCl_3$, 600 MHz) of ditryptophenaline (**136**)

The molecular weight of **136** was deduced from the ESI mass spectrum, which showed *m/z* 715 $[M+Na]^+$, 691 $[M-H]^-$ corresponding to *m/z* 692. The HRESIMS established the empirical molecular formula as $C_{42}H_{40}N_6O_4$, which corresponded to ditryptophenaline (**136**).[218] This dimeric diketopiperazine was isolated previously from *Aspergillus flavus* [219], as well as isolated recently from a marine-derived fungus *Aspergillus flavus* C-F-3.[56] The total synthesis of ditryptophenaline was achieved by Movassaghi *et al*.[220]



136

Table 18: ^{13}C and ^{1}H NMR spectroscopic data for ditryptophenaline (**136**) in $CDCl_3$

Position	δ_C [a] mult	δ_H [b] (mult.; *J* in [Hz])
1	165.3, C_q	-
2	163.9, C_q	-
3	150.1, C_q	-
4	134.4, C_q	-
5	129.5, CH	7.03 (t, 7.80 Hz)
6, 7	129.3, 2CH	7.10 (d, 8.30Hz)
8, 9	129.2, 2CH	7.51 (m)
10	127.8, CH	7.45 (m)
11	126.4, C_q	-
12	125.6, CH	6.93 (d, 7.5 Hz)
13	118.8, CH	6.66 (t, 7.5 Hz)
14	109.5, CH	6.51 (d, 7.8 Hz)
15	78.6, CH	4.78 (s)
16	63.0, CH	4.79 (s)
17	58.9, CH	4.21 (dd, 4.7, 7.3)
18	58.5, C_q	-
19	36.1, CH_2	3.48 (dd, 3.1, 14.3 Hz) 3.20 (dd, 4.4, 14.3 Hz)
20	35.9, CH_2	1.98 (dd, 4.9, 12.3 Hz) 1.53 (t, 12.2 Hz)
21	32.5, CH_3	2.98 (s)
-	-	NH, 4.75 (s br)

[a] ($CDCl_3$, 300 MHz), [b] ($CDCl_3$, 125 MHz)

4.19 Endophytic fungus *Aspergillus fumigatus*

The endophytic isolate *Aspergillus fumigatus* R7 was selected according to the chemical and biological screening. The TLC analysis of the crude extract exhibited different coloured reactions with anisaldehyde/sulphuric acid; the extract showed good biological activities against different microorganisms Figure 259.

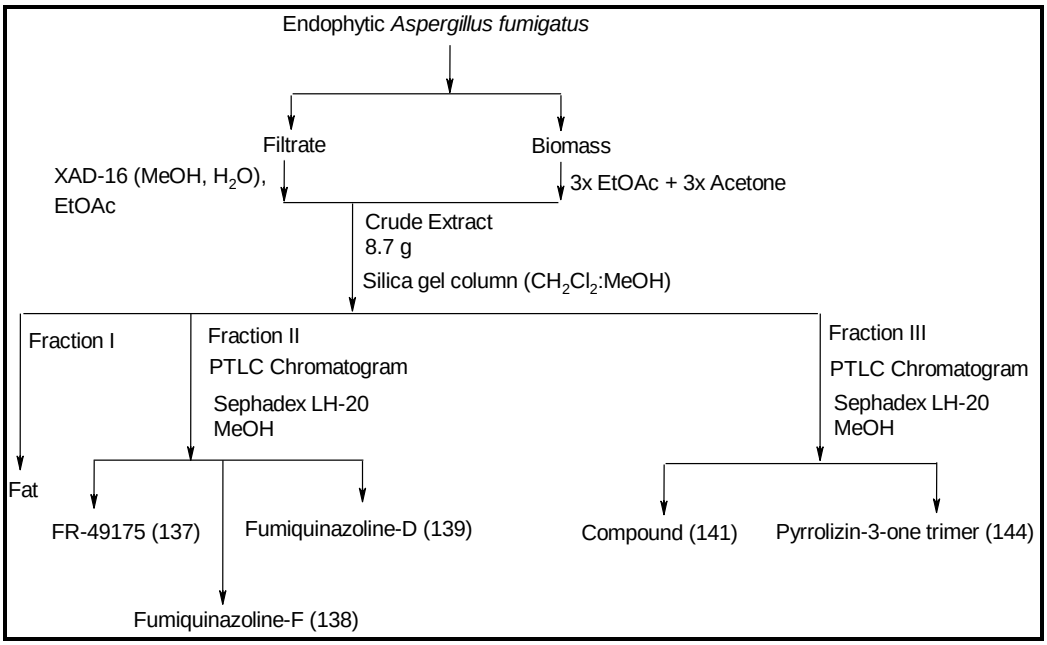


Figure 237: Work-up scheme of endophytic fungus *Aspergillus fumigatus*

4.19.1 FR-49175

FR-49175 (**137**) was isolated as colourless slightly UV absorbing oil, which turned brownish with anisaldehyde/sulphuric acid and heating. The ^{1}H NMR spectrum showed in the olefinic region two multiplets at δ 5.97 and 5.91, and a doublet at δ 5.66. The chemical shift and multiplicity suggested an allylic pattern. In addition, two oxymethine protons overlapped at δ 4.92. Moreover, the AB signal of an oxymethylene group was found at δ 4.24 and 3.85; a further methylene AB signal appeared at δ 3.11 and 2.92 and was overlaid with a methyl singlet at δ 3.10. Finally two methyl singlets were seen at δ 2.26 and 2.23.

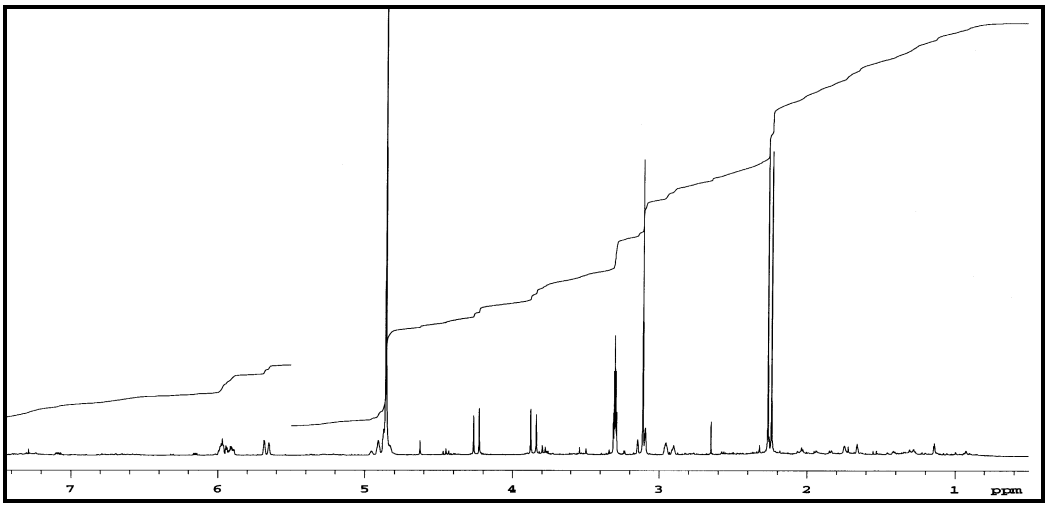


Figure 238: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of FR-49175 (**137**)

The ^{13}C NMR spectrum of FR-49175 (**137**) presented 15 carbon signals, including two carbonyls at δ 168.5 and 167.8 for two amide carbons of a piperazinedione moiety, and a quaternary carbon at δ 134.1. In addition, three sp^2 methine carbons at δ 130.8, 124.8 and 120.8 were observed. In the aliphatic region, two quaternary carbons at δ 74.4 and 73.2 along with two methine signals at δ 75.8 and 70.3 were seen, in addition to two methylenes at δ 64.7 and 39.7. Finally three methyl signals at δ 29.1, 15.2 and 13.5 were observed.

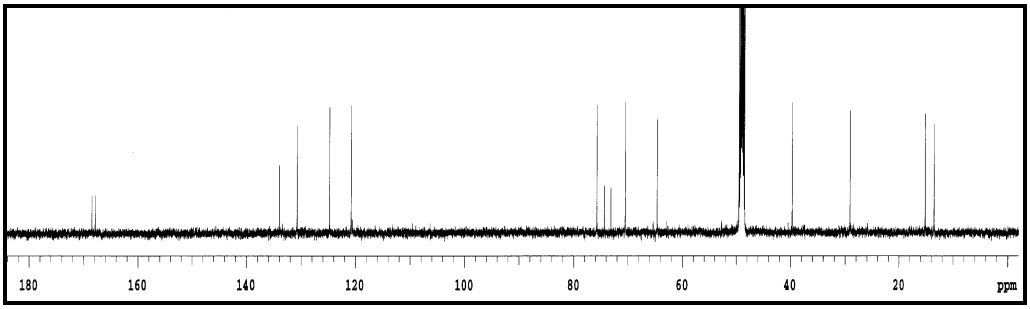
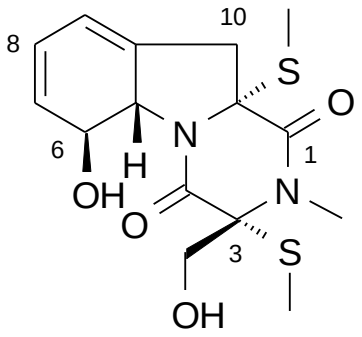


Figure 239: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of FR-49175 (**137**)

The ESI mass spectrum **137** afforded a *pseudo*molecular ion at 379 $[M+Na]^+$, 735 $[2M+Na]^+$, which gave the molecular weight of 356 Dalton; HRESIMS revealed the molecular formula as $C_{28}H_{44}O_9$. A search in AntiBase and the Chemical Abstracts using above spectroscopic data confirmed the structure as FR-49175 (**137**).



137

Table 19: ^{13}C and ^{1}H NMR spectroscopic data for FR-49175 (**137**) in CD_3OD

No.	*δc*, mult.	δ_H (mult.; *J* in [Hz])
1	167.8, C_q	-
3	74.4, C_q	-
4	168.5, C_q	-
5a	70.3, CH	4.92 (m, 2H overlapped)
6	75.8, CH	4.92 (m, 2H overlapped)
7	130.8, CH	5.97 (m)
8	124.8, CH	5.91 (m)
9	120.8, CH	5.66 (d, 9. 7)
9a	134.1, C_q	-
10	39.7, CH_2	3.11 (d, 15.7)
		2.92 (dd, 2.4, 19.3)
10a	73.2, C_q	-
2-NCH_3	29.1, CH_3	3.10 (s)
3-SCH_3	13.5, CH_3	2.26 (s)
3-OCH_2	64.7, CH_2	4.24 (d, 11.5)
		3.85 (d, 9.7)
10a- SCH_3	15.2, CH_3	2.23 (s)

4.19.2 Fumiquinazoline-F

Fumiquinazoline-F (**138**) was isolated as colourless UV absorbing solid, which turned yellow with anisaldehyde/sulphuric acid and heating. The molecular weight was obtained from the ESI mass spectrum, which showed *pseudo*-molecular ion peaks at *m/z* 357 $[M-H]^-$, 381 $[M+Na]^+$, 739 $[2M+Na]^+$, and 1097 $[3M+Na]^+$, corresponding to the molecular weight of 358 Dalton. HRESIMS established the molecular formula as $C_{21}H_{18}N_4O_2$. The ^{1}H NMR spectrum of **138** displayed in the aromatic

region 8 proton signals separated as two groups, namely 2H doublets of doublets at δ 8.28 and 7.79 and two proton signals overlapped at δ 7.54, indicating a 1,2-disubstituted benzene ring. Another group appeared as two doublets at δ 7.26 (J = 8.2) and 7.14 (J = 8.0) and further two protons appeared as triplets of doublet at δ 6.98 (J = 7.1, 1.1) and 6.69 (J = 7.8, 0.7), which gave another 1,2-disubstituted ring. A singlet overlapped at δ 6.76 with the integration of one proton, which was characteristic of the indole moiety. Additionally, the spectrum exhibited two 1H signals as doublets of doublets at δ 5.50, indicating probably methines attached to oxygen. Another one was displayed at δ 2.96; moreover, methylene signals of an ABX system appeared at δ 3.63 and 3.55. Finally, a methyl doublet at δ 1.28 attributed to the fragment CH_3CH-O was observed.

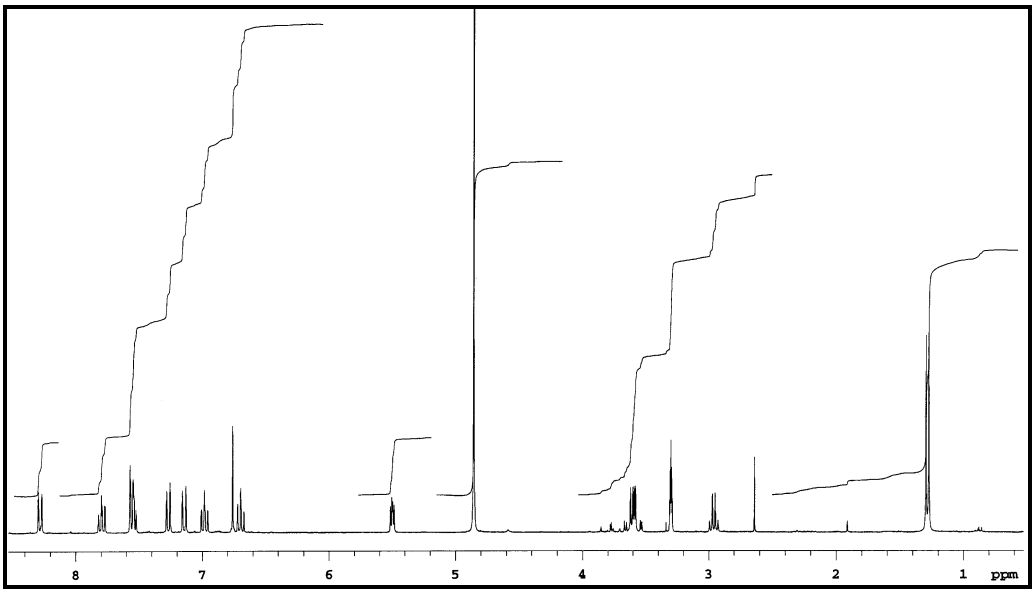


Figure 240: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of Fumiquinazoline F (**138**)

According to the formula, the ^{13}C NMR spectrum revealed 21 carbon signals, including two amide carbonyl signals at δ 171.0 and 162.5, two quaternary sp^2 carbons at δ 153.6 and 148.6 attached to heteroatoms and 12 aromatic carbons between δ 136.8-109.3. In addition, two oxymethine carbons at δ 59.1 and 50.3, a methylene carbon at δ 27.8 and a methyl carbon at δ 18.6 were observed.

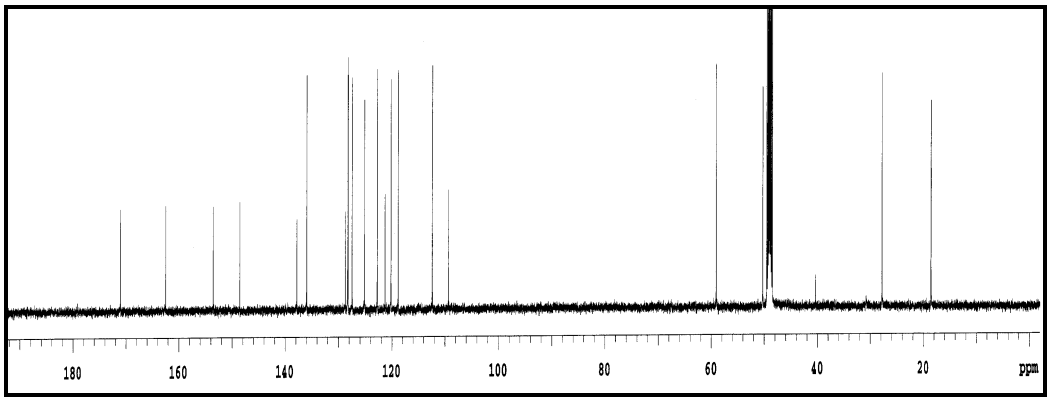
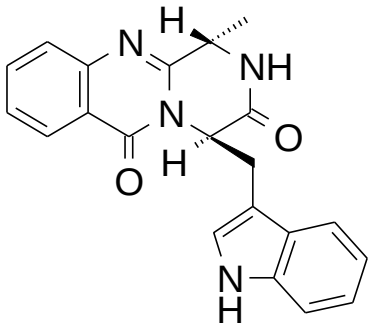


Figure 241: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of fumiquinazoline F (**138**)

A search in AntiBase[77] and the Chemical Abstracts using the spectroscopic data above confirmed the structure as fumiquinazoline F (**138**)**.**[221] This compound had been isolated from the fungus *Aspergillus fumigatus*, which was isolated from the marine fish *Pseudolabrus japonicus*[221]. Fumiquinazoline F (**138**) and C were also isolated from *Aspergillus lentulus*,[222] *Penicillium thymicola*,[223] and *Penicillium corylophilum*;[224] they showed high antitumor activity.[225,226] The synthesis had been published by Liu[227] and Wang.[228]



138

Table 20: ^{13}C and ^{1}H NMR spectroscopic data of fumiquinazoline F (**138**) in CD_3OD

No.	*δc*, mult.	δ_H (mult.; *J* in [Hz])
1	171.0, C_q	-
3	50.3, CH	2.96 (q, 6.7)
4	153.6; C_q	-
6	148.6, C_q	-
7	128.3, CH	7.54 (2H overlapped)
8	136.0, CH	7.79 (dd, 7.8, 1.5)
9	128.2, CH	7.54 (2H overlapped)
10	127.5, CH	8.28 (dd, 8.5, 1.8)
11	121.3, C_q	-
12	162.5, C_q	-
14	59.1, CH	5.50 (dd, 5.1, 3.4)
15	27.8, CH_2	3.63 (ABX, 14.8, 5.2)
		3.55 (ABX, 14.8, 3.4)
16	18.6, CH_3	1.28 (d, 6.7)
17	109.3, C_q	-
18	125.2, CH	6.76 (s)
20	137.8, C_q	-
21	112.4, CH	7.26 (d, 8.2)
22	122.8, CH	7.14 (d, 8.0)
23	120.2, CH	6.98 (td, 7.1, 1.1)
24	118.8, CH	6.69 (td, 7.8, 0.7)
25	128.7, C_q	-

4.19.3 Fumiquinazoline D

Fumiquinazoline D (**139**) was isolated from a UV absorbing zone, which turned yellow with anisaldehyde reagent and heating. It was purified by PTLC on silica gel followed by Sephadex LH-20 eluted by MeOH. ESIMS of fumiquinazoline D afforded *pseudo*molecular ion peaks at *m/z* 466 $[M+Na]^+$, 909 $[2M+Na]^+$, 1352 $[3M+Na]^+$, 442 $[M-H]^-$, and 885 $[2M-H]^-$, which gave a molecular weight of 443 Dalton. The HRESI mass spectrum established the molecular formula $C_{24}H_{21}N_5O_4$. The ^{1}H NMR spectrum showed two 1,2-disubstituted benzene rings: the first gave signals in the range of δ_H 8.18-7.52, while the signals of the second ring appeared between δ 7.42-7.16. In addition three signals at δ_H 5.66 (H-18), 5.45 (H-14) and

4.24 (H-20) indicated protons, which could be attached to heteroatoms or sp^2 carbons; additionally methylene signals at δ_H 3.16 and 2.21 were observed. Finally, a 3H singlet at δ 2.07 and a doublet at δ 1.08 were seen.

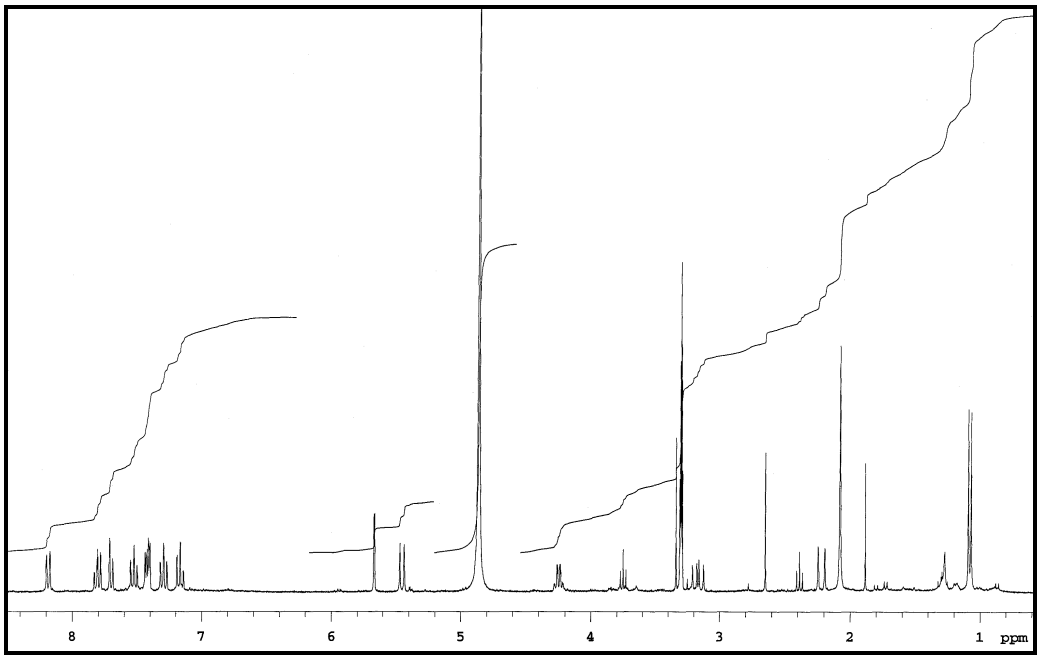


Figure 242: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of fumiquinazoline D (**139**)

In the ^{13}C NMR spectrum, 21 carbon signals were visible, as expected from the formula. Three amide carbons at δ 174.0, 171.1 and 162.5, thirteen sp^2 carbon signals at δ 154.3-116.3 were observed, along with two quaternary carbons at δ 84.5 and 72.1 attached to electron withdrawing atoms. Additionally, an sp^3methine carbon at δ 87.2, two methine carbons at δ 60.3, 54.6, methylene carbon at δ 44.5 and two methyl carbons signal at δ 19.0, 18.0 were seen.

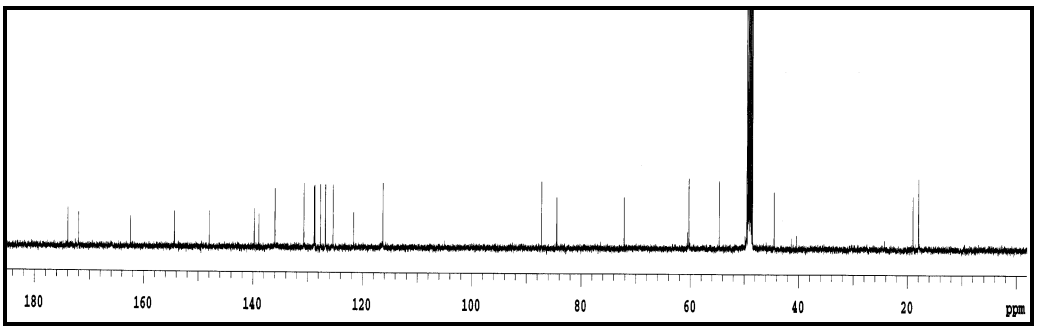
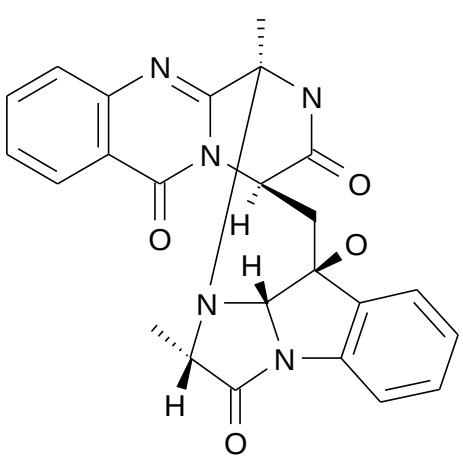


Figure 243: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of fumiquinazoline D (**139**)

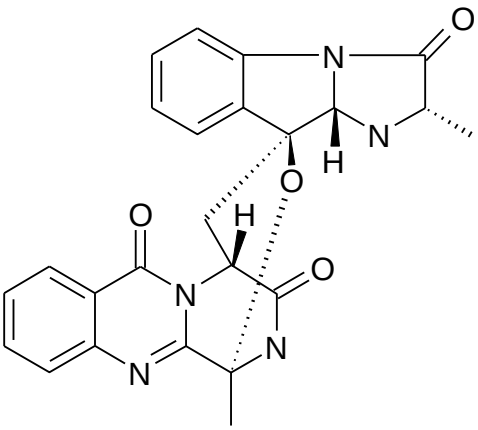
A search in AntiBase[77] and the Chemical Abstracts using above spectroscopic data resulted in two compounds; fumiquinazoline D (**139**) and its stereoisomer, fumiquinazoline C (**140**). The structure of fumiquinazoline D (**139**) was confirmed by the big difference of the chemical shift for C-3 between fumiquinazoline-D (**139**) and fumiquinazoline C (**140**). So the compound was identified as fumiquinazoline D (**139**).

Table 21: ^{1}H NMR and ^{13}C NMR shifts of fumiquinazoline D (**139**) (300, 125 MHz) in CD_3OD

No.	*δc*, mult.	δ_H (mult.; *J* in [Hz])
1	174.0, C_q	-
3	72.1, C_q	-
4	154.3; C_q	-
6	148.0, C_q	-
7	136.0, CH	7.69 (dd, 8.1, 1.1)
8	127.6, CH	7.80 (td, 8.4, 1.5)
9	128.8, CH	7.52 (td, 8.2, 1.2)
10	128.7, CH	8.18 (dd, 8.0, 1.5)
11	121.3, C_q	-
12	162.5, C_q	-
14	54.6, CH	5.45 (d, 10.7)
15	44.5, CH_2	3.16 (m, 10.3)
		2.21 (dd, 15.7, 0.8)
16	19.0, CH_3	2.07 (s)
17	84.5, C_q	-
18	87.2, CH	5.66 (d, 1.5)
20	60.3, CH	4.24 (q, 6.4, 1.7)
21	171.1, C_q	-
23	139.0, C_q	-
24	116.3, CH	7.40 (m)
25	130.7, CH	7.29 (td, 1.21, 7.5),
26	126.7, CH	7.16 (td, 1.13, 7.5)
27	125.3, CH	7.42 (m)
28	139.8, C_q	-
29	18.0, CH_3	1.08 (d)



139



140

4.19.4 (Z,Z)-N,N'-[1-[(4-Hydroxyphenyl)methylene]-2-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-formamide

Compound **141** was isolated as a colourless UV absorbing solid, which turned violet on spraying with anisaldehyde reagent and heating. ESIMS of compound **141** afforded *pseudo*molecular ion peaks at *m/z* 361 $[M+Na]^+$, and 699 $[2M+Na]^+$, which gave a molecular weight of 338 Dalton. The HRESI mass spectrum established the molecular formula $C_{19}H_{18}N_2O_4$. Compound **141** was isolated as a mixture of two isomers according to two unsaturated double bonds in the molecule. The ^{1}H NMR spectrum revealed an exchangeable acidic proton at δ 9.64 of a hydroxyl or amide group. At δ 9.54-9.40 and 9.37-9.24, *cis* and *trans* amide protons were visible, respectively. Overlapping signals appeared at δ 7.5-7.3 (H-4/H-4') and δ 6.9-7.0/6.7-6.8 (H-5/H-5'). Finally in the aliphatic region two singlet signals overlapping at δ 3.77 and 3.76 for methoxy groups were observed.

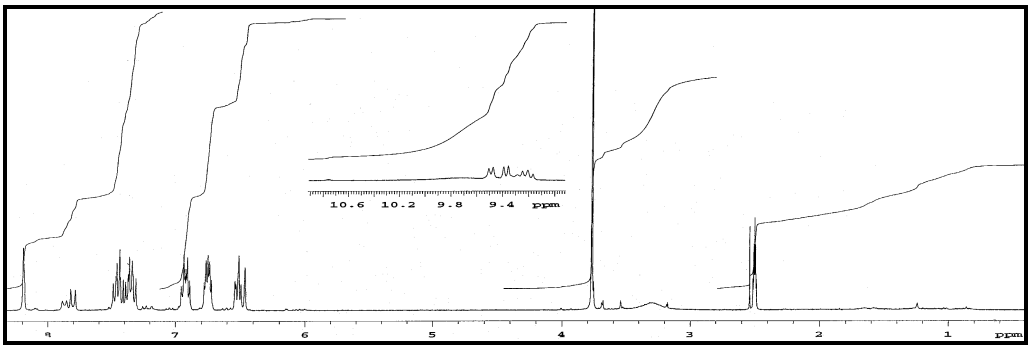


Figure 244: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of compound **141**

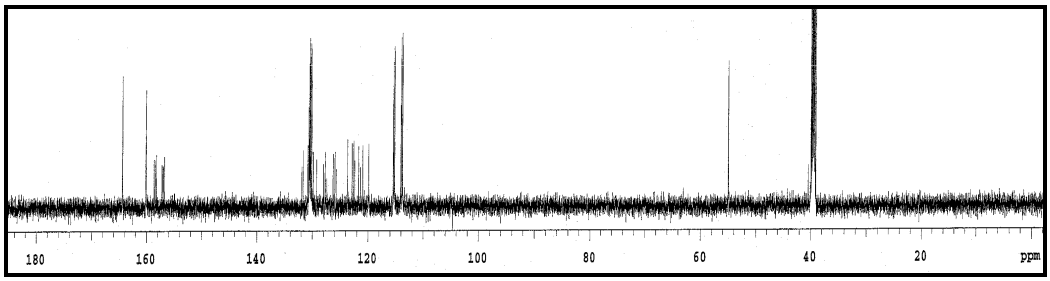
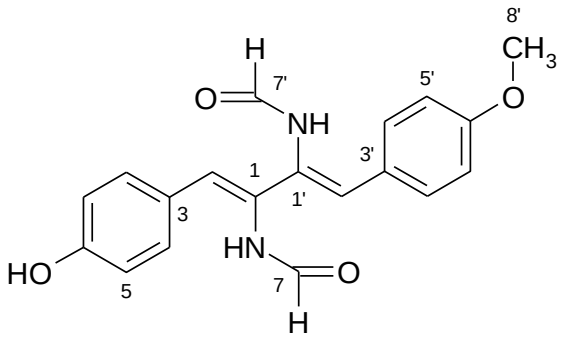


Figure 245: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **141**



141

Table 22: ^{13}C NMR shifts of compound **141** (125 MHz, DMSO-d_6) in comparison with literature values

	Compound (**141**)	Lit.[229] (**141**)
Position	δ_C	δ_C
1	131.9, 129.9	131.9, 129.9
1'	131.7, 129.3	131.7, 129.3
2	121.7, 121.4, 121.0, 119.9	121.7, 121.4, 121.0, 120.0
2'	123.0, 122.9, 122.6, 122.4	123.7, 122.9, 122.6, 122.4
3	126.0, 126.3, 126.0, 125.7	126.4, 126.1, 126.1, 125.8
3'	128.1, 127.8, 127.7,127.4	128.1, 127.8, 127.7, 127.5
4	130.6	130.6
4'	130.5	130.5
5	114.1, 114.0, 113.8, 113.7	114.1, 114.0, 113.9, 113.8
5'	115.5, 115.4, 115.3, 115.2	115.5, 115.4, 115.3, 115.2
6	157.2, 157.1, 157.0, 156.8	157.2, 157.0, 157.0, 156.8
6'	158.7, 158.6, 158.4, 158.3	158.7, 158.5, 158.5, 158.3
7	164.4, 164.4	164.5, 164.5
7'	160.2, 160.1	160.2, 160.2
8	-	-
8'	55.1	55.1

Compound **141** was previously isolated from the fungus *Hamigera avellanea* and exhibited a marginal activity against a variety of pathogenic fungi and bacteria[229]. According to a survey in literature, many secondary metabolites such as xanthocillin-X, contain the isocyanide moiety is a curious structural feature. It is most likely that biosynthesis, in these examples, involves formal quenching of a carbonium ion by addition of a cyanide ion.[230]

4.19.5 Pyrrolizin-3-one trimer

The pyrrolizin-3-one trimer **144** was isolated as colourless solid from a UV absorbing band, which turned to yellow with anisaldehyde/sulphuric acid and heating. The ESI mass spectrum displayed $[2M+Na]^+$ and $[M+Na]^+$ ion peaks at *m/z* 749 and 386 in positive mode, which fixed the mass as 363 Dalton. The odd mass number was an indication of an odd number of nitrogen atoms in the molecular formula. HRESIMS revealed the molecular formula as $C_{21}H_{21}N_3O_3$. The ^{1}H NMR spectrum of **144** exhibited four proton signals in the olefinic region, including three doublet of doublet signals at δ 6.20 (J = 3.1, 0.8), 5.88 (J = 3.1, 1.2), 5.80 (J = 3.2, 1.4), and a remaining doublet proton at δ 6.24 (J = 3.1). The chemical pattern and coupling constant suggested protons of a five-membered heterocycle. In the aliphatic region eight methylene multiplets appeared, among them broad singlets at δ 3.03 and 2.95 integrating for four protons. In addition a methine signal of a proton attached to a heteroatom appeared at δ 3.87.

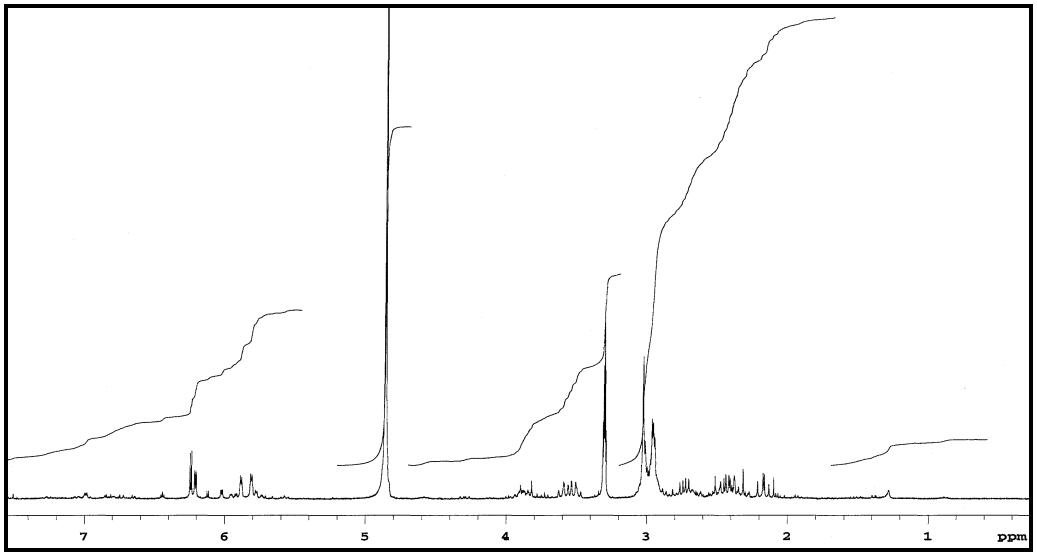


Figure 246: ^{1}H NMR spectrum (CD_3OD, 300 MHz) of pyrrolizin-3-one trimer (**144**)

The ^{13}C NMR spectrum exhibited 21 carbons signals, among them three carbons for amides, acids or esters at δ 177.8, 175.0 and 174.4, four quaternary carbons, and four methine sp^2 signals. In the aliphatic region eight methylene groups along with one quaternary carbon and a methine carbon were visible.

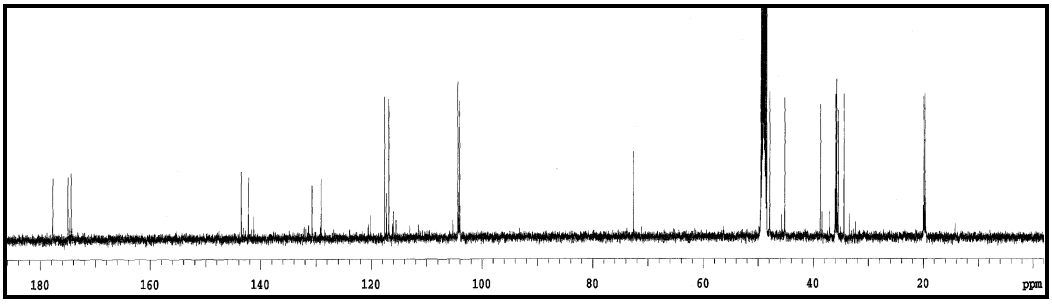


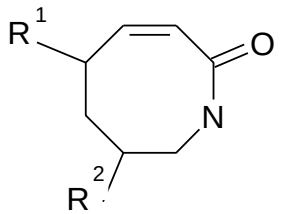
Figure 247: ^{13}C NMR spectrum (MeOH, 125 MHz) of pyrrolizin-3-one trimer **144**

Table 23: Comparison of the ^{1}H NMR and ^{13}C NMR data of the pyrrolizin-3-one trimer **144** with literature values[231]

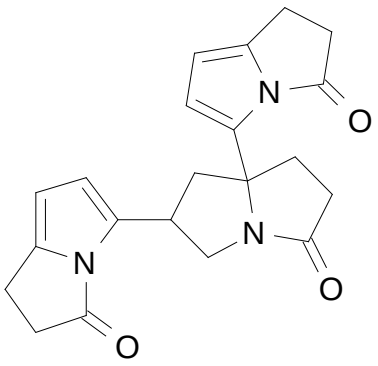
	Trimer **144**		Trimer **144** (lit[231])	
Position	δ_C	δ_H (mult.; *J* in [Hz])	δ_C	δ_H (mult.; *J* in [Hz])
2	177.8, C_q	-	175.4	-
3	34.5, CH_2	2.70 (m, 3a)	33.5	2.70 (m, 3a)
		2.51-2.31 (m, 3b)		2.5-2.3 (m, 3b)
4	35.5, CH_2	2.51-2.31 (m)	34.8	2.5-2.3 (m)
5	72.6, C_q	-	70.0	-
6	45.2, CH_2	2.73 (m), 2.15 (dd, 11.9, 3.1)	44.6	2.78 (dd, 12.4, 6.6), 2.10 (dd, 11.6, 0.6)
7	36.0, CH	3.87 (m)	37.2	3.95 (m)
8	47.9, CH_2	3.55 (m)	46.8	3.63 (m)
2'	175.0, C_q	-	172.6	
3'	35.8, CH_2	2.95 (m)	35.1	2.93 (s br)
4'	19.6, CH_2	2.95 (m)	19.0	2.93 (s br)
5'	142.2, C_q	-	139.9	
6'	104.4 CH	5.80 (dd, 3.2, 1.4)	103.6	5.78 (dd, 3.1, 1.5)
7'	116.9 CH	6.20 (dd, 3.1, 0.8)	115.3	6.14 (dd, 4.1, 0.9)
8'	129.1, C_q	-	128.5	
2"	174.4, C_q	-	172.4	
3"	38.7 CH_2	3.03 (s br)	35.0	3.01 (s br)
4"	19.8 CH_2	3.03 (s br)	19.2	3.01 (s br)
5"	143.6, C_q	-	141.2	
6"	104.3 CH	5.88 (dd, 3.1, 1.2)	103.5	5.84 (dd, 3.1, 1.2)
7"	117.6 CH	6.24 (d, 3.1)	116.8	6.26 (d, 3.1)
8"	130.8, C_q	-	130.3	

The precursors of the pyrazolone-2-one trimer are azocin-2-one (**142**) derivatives, which are very rare in nature. However, some homologous caprolactams are known, such as caprolactin A and B [232]. Although the chemistry of caprolactam and its synthetic unsaturated derivatives has been explored extensively, very few reports appeared regarding the eight membered homologues, the 1H-azocin-2-ones[233]. The unusual reactivity of tetrahydro-1*H*-azocin-2-one (**143**) has been attributed to ring conformational effects, which promote an intramolecular cyclisation in suitably substituted tetrahydro-1*H*-azocin-2-ones.[233] Similarly, azocin-2-one (**142**) derivatives may be decomposed during the purification on silica gel or Sephadex LH-20 columns to afford the pyrrolizin-3-one trimer (**144)** as artefact.

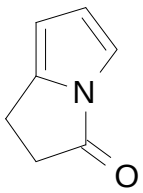
Fotso and Li have shown that the cyclisation of **142** in alcohol-free chloroform was catalysed by a light-induced liberation of HCl: Protonation of 5-OH will allow an attack of the nitrogen atom on C-5 under cyclisation and elimination of water. A second loss of water and rearrangement of the double bond delivers **145**. In the absence of chloroform solution of **145** no reaction occurred. This observation let to assume that the trimer **144** may result from an intermolecular reaction of **142** or between **142** and **145**.[231]



142: R^1 = OH, R^2 = OH
143: R^1 = H, R^2 = H



144



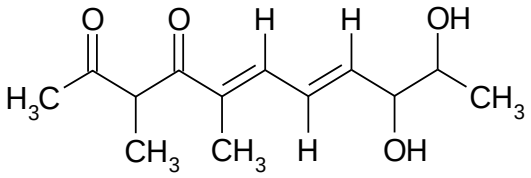
145

Summary

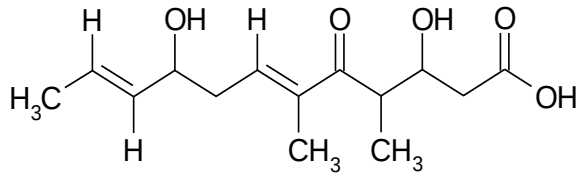
Natural products are still an important source for medical drugs. Amongst the micro-organisms, especially the genus *Streptomyces*, an important group of actinomycetes, is still in the focus of interest because their high productivity of a wide range of secondary metabolites. The characterization and identification of new microbial metabolites is still of high importance, and also the re-investigation of already identified molecules for new activities is still of interest. [234]

In the present work fifteen terrestrial *Streptomyces* spp., a *Bacillus* species and three fungal strains have been fermented in large-scale. The selection of the strains have been done according to their biological activities and results from the chemical screening. The successive steps for upscaling, extraction and separation were performed under standard conditions. Dereplication of isolated compounds was done with the help of AntiBase and for the structure elucidation, NMR, MS, and HPLCMS measurements were performed.

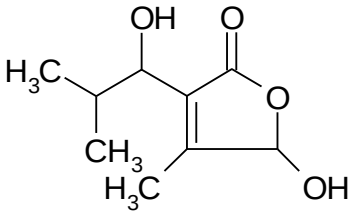
The crude extract of the terrestrial *Streptomyces* sp. ANK 264 showed activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and weak activity against *Artemia salina*. From a 25 l shaker culture, deferrioxamine E (**41**) and two new compounds, suhagcine I (**44**) and II (**45**) were isolated, together with known compounds such as metabolite VIIb (**40**), pentenomycin I (**42**), 5-hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2-(5H)furanone (**43**) and 2, 5-furandimethanol (**46**), in addition to juglorescein (**38**).



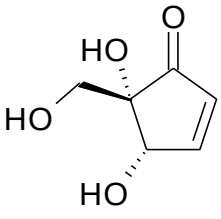
(**44**)



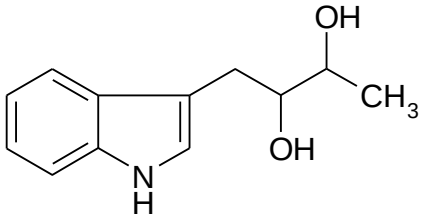
(**45**)



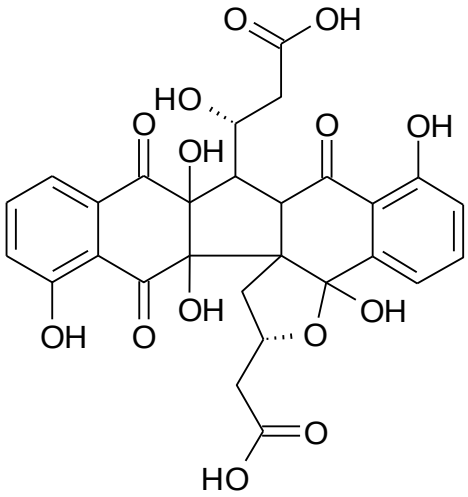
(**43**)



(**42**)

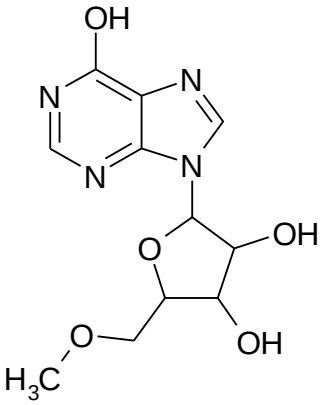


(**40**)

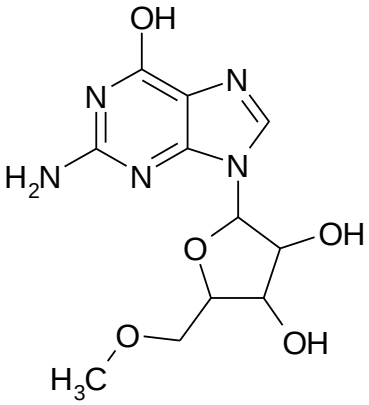


(**38**)

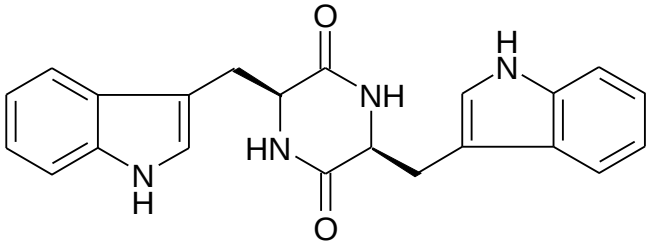
In the pre-screening, the crude extract of the terrestrial *Streptomyces* sp. ANK 251 showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and weak activity against *Artemia salina*. A 25 l shaker culture delivered two new nucleosides as colourless solids, namely 5'-methoxyinosine (**48**) and 5'-methoxyguanosine (**49**) as well as virginiae butanolide F (**47**) and fellutanine A (**50**).



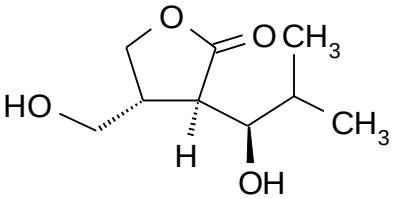
(**48**)



(**49**)



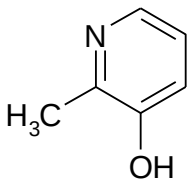
(**50**)



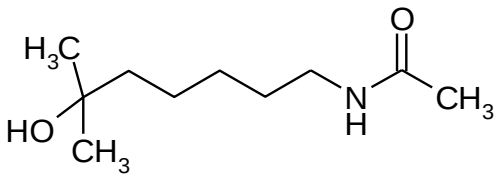
(**47**)

The terrestrial *Streptomyces* sp. ANK 275 showed antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphy-*

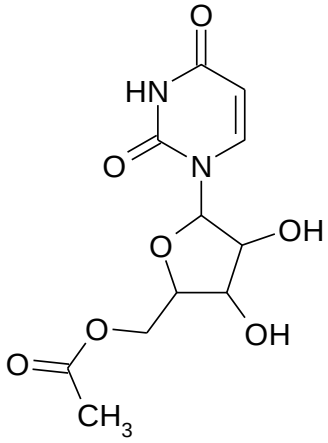
lococcus aureus, Mucor miehei and *Artemia salina*. Purification of the crude extract obtained from a 25 l shaker culture produced succinic acid, vanillic acid and N-(6-hydroxy-6-methyl-heptyl)-acetamide (**56**), as well as two new nucleosides, 5'-acetyluridine (**58**) and 5'-acetyl-2'-deoxy thymidine (**59**) and 2-methylpyridine-3-ol (**55**), which is new as microbial product.



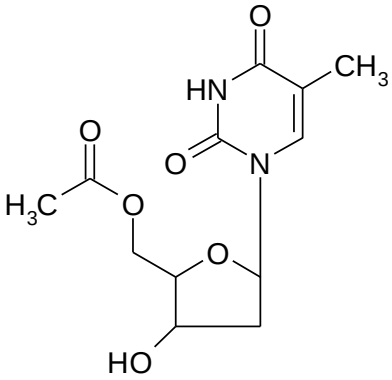
(**55**)



(**56**)

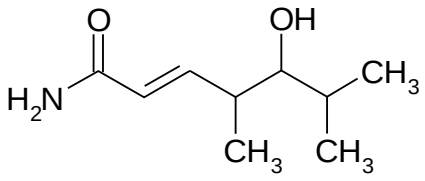


(**58**)

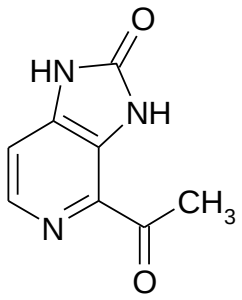


(**59**)

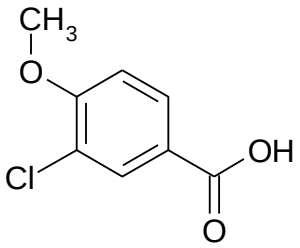
The crude extract of the terrestrial *Streptomyces* sp. ANK 275 afforded (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**) as a new natural product, which is a sub-structure of ostreogrycin A. In addition, five known compounds, namely 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**63**), pyrrole-2-carboxamide (**67**), indole-3-acetic acid, 4-hydroxy benzylamine (**64**) and 3-chloro-4-methoxybenzoic acid (**66**) were isolated.



(**60**)

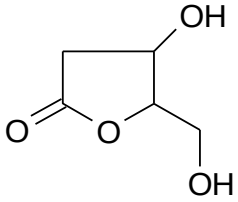


(**63**)

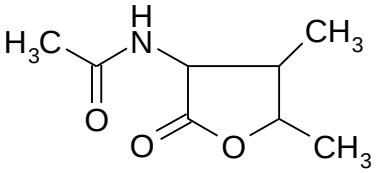


(**66**)

The chemical screening of the crude extract of the terrestrial *Streptomyces* ANK 312 showed two UV absorbing bands, which turned blue with anisaldehyde/sulphuric acid on TLC. A 25 l shaker culture delivered the γ-lactone **68** and N-(4,5-dimethyl-2-oxo-tetrahydro-furan-3-yl)-acetamide (**69**) which were known from plants but are isolated here for first time from bacterial strains.

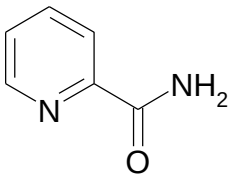


(**68**)

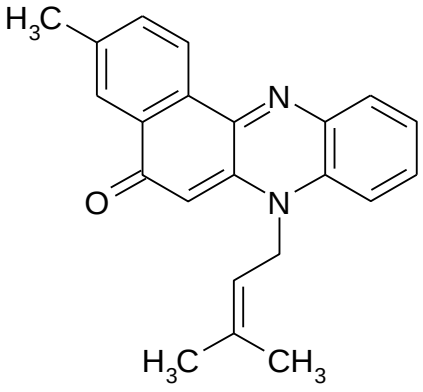


(**69**)

The extract of the terrestrial *Streptomyces* sp. ANK 320 showed weak biological activity against microorganisms (*Mucor miehei* and *Artemia salina*). In a series of chromatographic steps, the crude extract gave two new compounds, namely chromo-phenazine A (**74**) and picolinamide (**76**) as well as phenazine-1-carboxamide.



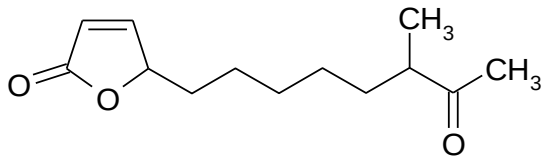
(**76**)



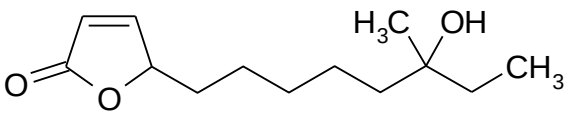
(**74**)

The terrestrial *Streptomyces* sp. isolate ADM 9 exhibited a biological activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and high activity against *Artemia salina*. Investigation of the extract yielded known compounds, namely 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**77**),

4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**78**), tryptophol, 4-hydroxy benzoic acid, indole-3-carboxylic, and ferulic acid (**79**).

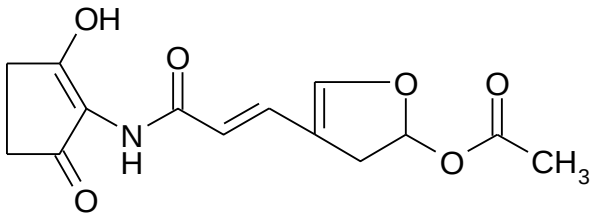


(**77**)

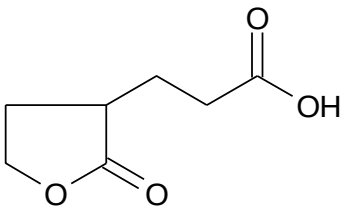


(**78**)

The crude extract of the terrestrial *Streptomyces* sp. ANK 179 was found to be moderately active against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and *Artemia salina*. The chemical investigation afforded reductiomycin (**80**) along with the new 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**).

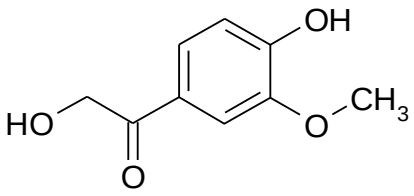


(**80**)

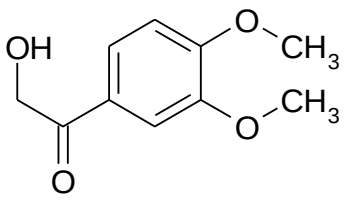


(**81**)

The extract of the terrestrial *Streptomyces* sp. ANK 174 showed in the agar diffusion test activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and moderate activity against *Artemia salina*. A 25 l shaker culture of the strain delivered two new natural compounds, namely hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**) and 2-hydroxy-1-(3,4-dimethoxy-phenyl)-ethanone (**85**), respectively, in addition to lactone R4 (**82**) and PHB.



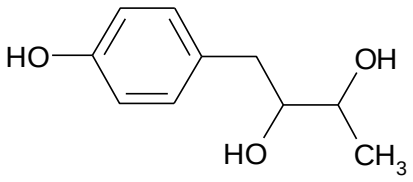
(**84**)



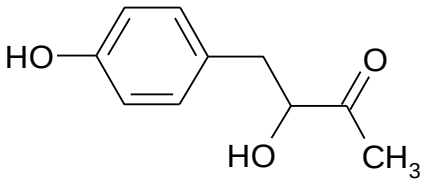
(**85**)

The biological screening of the terrestrial *Streptomyces* sp. WO 990 indicated activity against *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylo-*

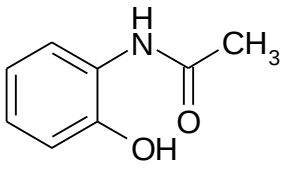
coccus aureus, Mucor miehei and *Artemia salina*. Purification of the extract resulted in the isolation of two new natural compounds, 1-(4-hydroxy-phenyl)-butane-2,3-diol (**86**) and 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**), along with 3-indolylglyoxylamide (**93**) and N-acetyl-2-aminophenol (**92**), as well as several trivial compounds.



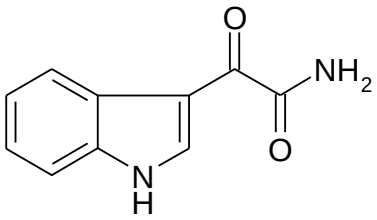
(**86**)



(**89**)

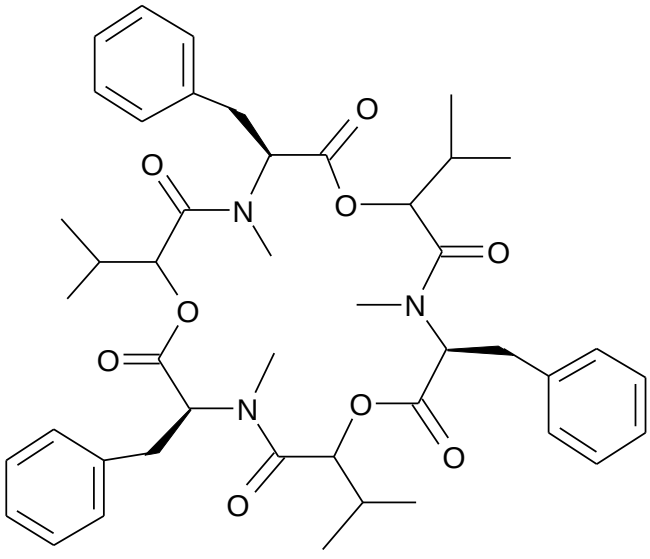


(**92**)

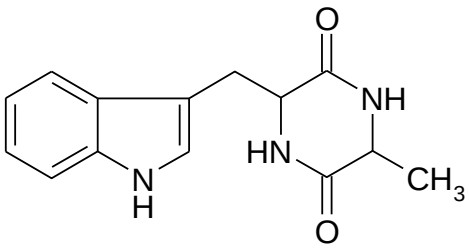


(**93**)

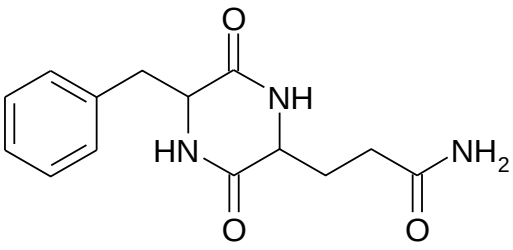
From bacterial strains investigated in a cooperation with Jana Tiefenau (University of Braunschweig) many diketopiperazines were isolated, among them the new natural product *cyclo*(Phe,Glu) (**104**) and known compounds including Beauvericin (**98**) and *cyclo*(Tyr,Pro) (**116**), *cyclo*(Pro,Val), *cyclo*(Ala,Pro) (**106**), *cyclo*(Dehydroala,Ile) (**113**), *cyclo*(Ala,Try) (**99**), *cyclo*(Ser,Try) (**100**), and *S*-methyl-adenosine (**102**).



(**98**)

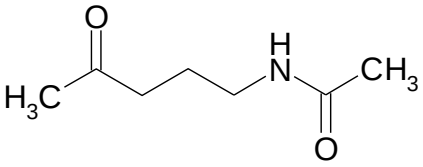


(**99**)

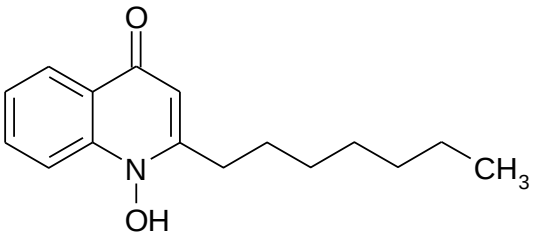


(**104**)

The extract of *Bacillus subtilis* MZ 6 was selected for further investigation because of its activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes (*Tü57), *Staphylococcus aureus*, and *Artemia salina*. N-(4-Oxo-pentyl)-acetamide (**107**) was isolated as new natural product from a 25 l shaker culture. Additionally, four known compounds were isolated, *cis-cyclo*(Ala,Pro), acetyltryptamine (**108**), tryptophane, and finally KF8940 (**110**).

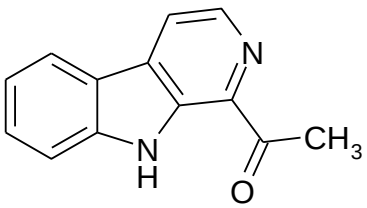


(**107**)

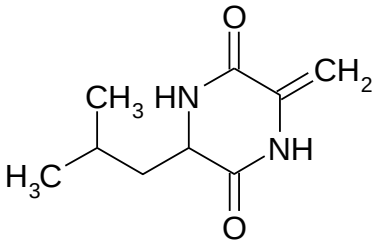


(**110**)

The extract of the terrestrial *Streptomyces* sp. N859 afforded seven known compounds including 1-acetyl-*β*-carboline (**111**), *cyclo*(Dehydroala,Leu) (**113**), as well as *cyclo*(Ala,Ile) (**114**), *trans-cyclo*(Tyr,Pro) (**115**), and *cis-cyclo*(Tyr,Pro) (**116**), 3-hydroxyacetylindole (**117**), indolyl-3-carboxylic acid and anthranilic acid. The crude extract showed no activity against microorganisms.



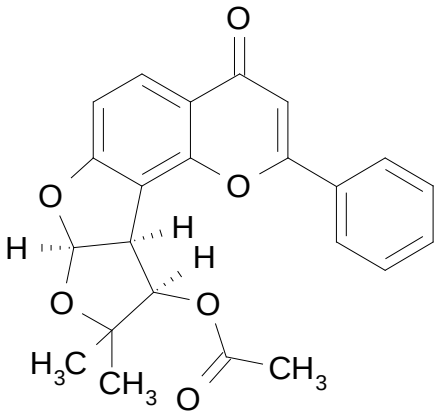
(**111**)



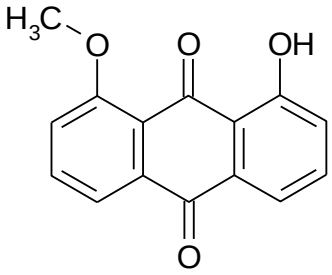
(**113**)

The extract of the marine-derived *Streptomyces* sp. B7547 was found to have antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*,

and moderate activity against *Artemia salina.* Separation afforded the plant metabolites pseudosemiglabrin (**118**) and semiglabrin; the latter one was obtained as a mixture with PHB. In addition, 1-hydroxy-8-methoxyanthraquinone (**119**), and a mixture of two epimeric glycosides (**120**) was isolated.

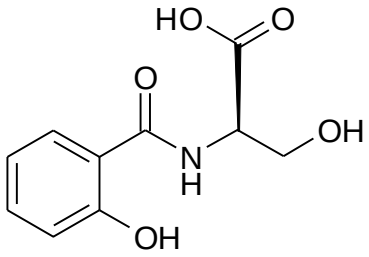


(**118**)

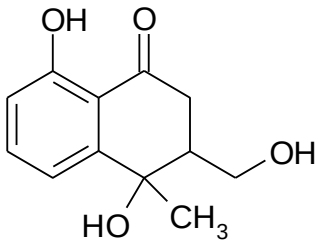


(**119**)

The extract of the terrestrial *Streptomyces* sp. GW 7/186 displayed a biological activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Mucor miehei* and *Artemia salina*. The isolation of the crude extract yielded to madurastatin B2 (**121**) and the tetralone **122**.

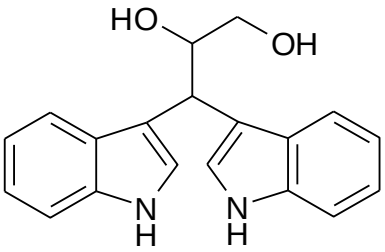


(**121**)

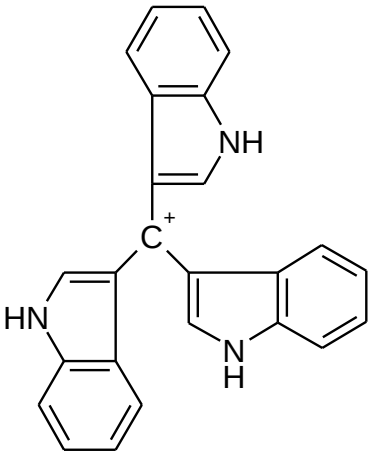


(**122**)

Cultivation of the terrestrial *Streptomyces* sp. MH4 in 25 l of M2 medium and separation of the crude extract gave 3-(3,3-bisindolyl)propane-1,2-diol (**125**) and turbomycin A (**126**) as well as trivial indole compounds together with nonactic acid (**123**) and homononactic acid (**124**).

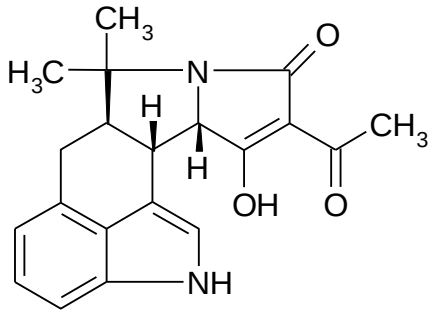


(**125**)



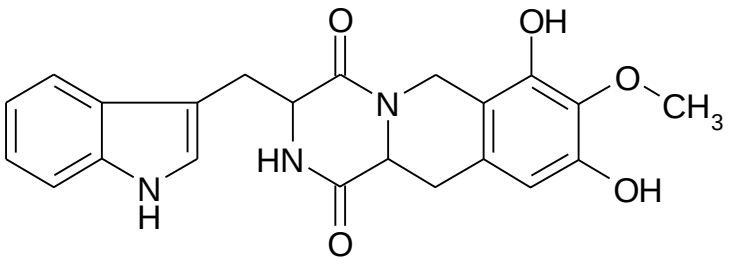
(**126**)

Fungi are rich sources of structurally unique and biologically active secondary metabolites,[235] and their metabolites may gain great importance in medical, industrial and/or agricultural application.[236] In the present work, two fungal strain were investigated according to their biological activities and the results of the chemical screening. A *Trichoderma* sp. showed activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger* in the agar diffusion test. From a 30L fermentor, four compounds were isolated, namely kojic acid (**127**), ergosterol (**128**), ergosterol peroxide (**129**) and α-cyclopiazonic acid (**130**).

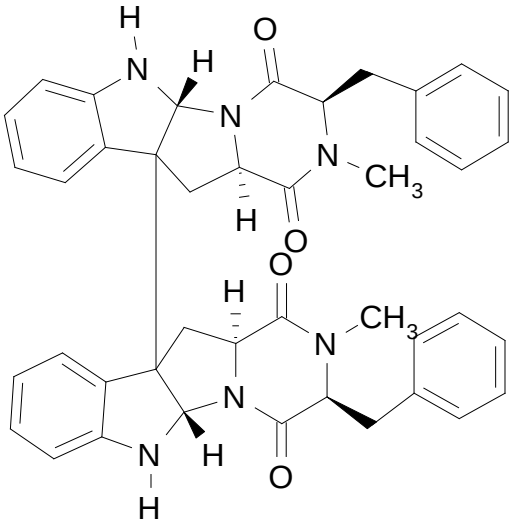


(**130**)

The fermentation of *Aspergillus oryzae* in a 30L scale afforded ditryptophenaline (**136**) and a new diketopiperazine, namely 7,9-Dihydroxy-3-(1H-indol-3-ylmethyl)-8-methoxy-2,3,11,11a-tetrahydro-6H-pyrazino[1,2-b]isoquinoline-1,4-dione (**134**).

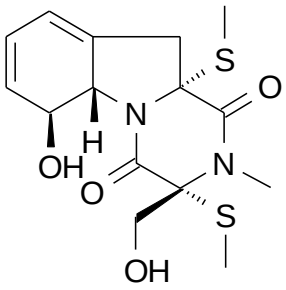


(**134**)

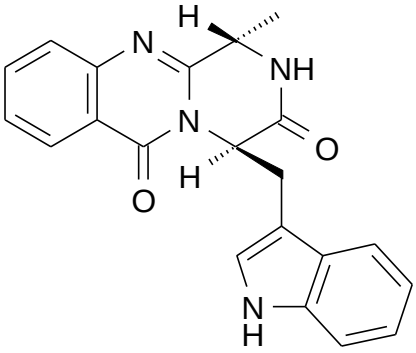


(**136**)

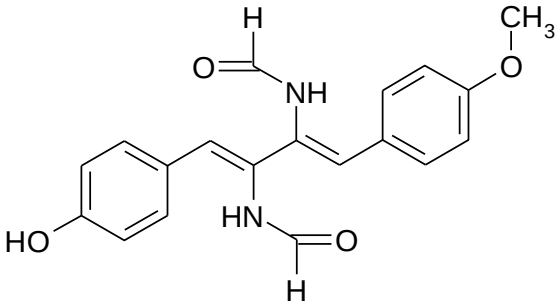
Endophytic fungi are a rich but nearly untapped source of bioactive compounds.[237,56] In the pre-screening, the crude extract of the endophytic strain R7 isolated in Egypt it was found to exhibit activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger*. The chromatographic purification of this strain resulted in the isolation of FR-49175 (**137**), fumiquinazoline F (**138**), fumiquinazoline D (**139**), isolated with a mixture of stereoisomers of N,N'-[1-[(4-hydroxyphenyl)methylene]-2-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-formamide (**141**) and pyrrolizin-3-one trimer (**144**).



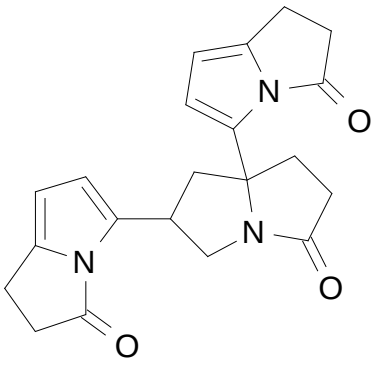
137



138



141



144

Table 24: Total number of isolated compounds from microorganisms in the present research work

Strains	No. of strains	Total no. of compounds	No of new compounds
Terrestrial *Streptomyces* sp.	14	45	14
Marine *Streptomyces* sp.	1	3	1
Bacillus sp.	2	10	2
Fungi	2	7	1
Endophytic fungi	1	5	-

The present work considered the terrestrial and marine *Streptomyces* sp. as target of pharmaceutical research due to their ability to be a rich source of bioactive compounds and interesting molecules.[238,239] Therefore research cooperation between chemists and biologists is important for further research into the discovery of new bioactive compounds.

5 Materials and Methods

5.1 General

UV/VIS spectra: Perkin-Elmer Lambda 15 UV/VIS spectrometer. - **Optical rotations:** Polarimeter (Perkin-Elmer, model 243), the concentrations were given in [mg/ml]. – **^{1}H NMR spectra:** Varian Unity 300 (300.145 MHz), Bruker AMX 300 (300.135 MHz), Varian Inova 500 (499.8 MHz), Varian Inova 600 (600 MHz). Coupling constants (*J*) in [Hz]. Abbreviations: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, br = broad. – **^{13}C NMR spectra:** Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz), Varian Inova 600 (150.7 MHz). Chemical shifts were measured relative to tetramethylsilane as internal standard. Abbreviations: APT (Attached Proton Test): CH/CH_3 up and C_q/CH_2 down. - **2D NMR spectra:** H,H COSY (^{1}H,^{1}H-Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Connectivity), HMQC (Heteronuclear Multiple Quantum Coherence) and NOSY (Nuclear Overhauser Effect Spectroscopy). - **Mass spectra**: EI MS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosine as standard. DCI-MS: Finnigan MAT 95 A, 200 eV, Reactant gas NH_3. ESI MS was recorded on a Finnigan LCQ

5.2 Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). – **Glass plates for chemical screening:** Merck silica gel 60 F254, (10 × 20 cm). - **Preparative thin layer chromatography (PTLC):** 55 g Silica gel P/UV_{254} (Macherey-Nagel & Co.) is added to 120 ml of demineralised water with continuous stirring for 15 minutes. 60 ml of the homogenous suspension is poured on a horizontal held (20 × 20 cm) glass plate and distributing the suspension covers the unfilled spaces. The plates were air dried for 24 hours and activated by heating for 3 hours at 130 °C. - **Column chromatography (CC):** MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co); silica gel (230-400 mesh) for flash chromatography: 30-60 μm (J. T. Baker); size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Amberlite XAD-16 resin was obtained from Rohm and Haas, France.

5.3 Spray Reagents

Anisaldehyde/sulphuric acid: 1 ml anisaldehyde was added to 100 ml of a stock solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid. **Ehrlich's reagent:** 1 g 4-dimethylaminobenzaldehyde was dissolved in a mixture of 25 ml hydrochloric acid (37%) and 75 ml methanol. It gives a red or violet colouration with indole and turns yellow with some other N-heterocycles.

Chlorine/*o*-dianisidin reaction: The reagent was prepared from 100 ml (0.032%) *o*-dianisidin in 1 N acetic acid, 1.5 g $Na_2WO_4 \cdot 2\ H_2O$ in 10 ml water, 115 ml acetone and 450 mg KI. The moistened TLC plate was kept ca. 30 min in a chlorine atmosphere (from 0.5 g $KClO_3$ + 2 ml conc. HCl) and then subjected to drying for ca. 1 h, till the excess of chlorine was evaporated and then dipped into the reagent. The reagent is specific for peptides as universal spraying reagent.

NaOH or KOH: 2 N NaOH or KOH solutions are used to identify *peri*-hydroxyquinones by deepening of the colour from orange to violet or blue.

5.4 Microbiological Materials

Storage of strains: Deep-freeze storage in a Dewar vessel, 1'Air liquid type BT 37 A. - **Capillaries for deep-freeze storage:** diameter 1.75 mm, length 80 mm, Hirschmann Laborgeräte Eberstadt. – Soil for soil culture**:** Luvos Heilerde LU-VOS JUST GmbH & Co. Friedrichshof (from the health shop). - **Ultraturrax:** Janke & Munkel KG. – **Shaker:** Infors AG (CH 4103 Einbach) type ITE. - **Laboratory shaker:** IKA-shaker type S50 (max. 6000 Upm). - **Autoclave:** Albert Dargatz Autoclave, volume 119 l, working temperature 121 °C, working pressure 1.2 kg/cm^2. - **Antibiotic assay discs:** 9 mm diameter, Schleicher & Schüll No. 321 261. - **Culture media:** glucose, bacto peptone, bacto agar, dextrose, soybean, mannit, yeast extract and malt extract were purchased from Merck, Darmstadt. - **Antifoam solution:** Niax PPG 2025; Union Carbide Belgium N. V. (Zwiijndrecht). – **Petri dishes:** 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – **Celite:** Celite France S. A., Rueil-Malmaison Cedex. - **Sterile filters:** Midisart 2000, 0.2 µm, PTFE-Filter, Sartorius, Göttingen. - **Laminar-Flow-Box:** Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1. - **Brine shrimp eggs** (***Artemia salina***)**:** SERA Artemia Salinenkrebseier, SERA Heinsberg (from aquaristic shops).

5.5 Recipes

All cultures were autoclaved at 1.2 bar and 120 °C. Sterilisation time for 1 l shaker culture: 33 min, 2 l concentrated medium for fermentor: 50 min and fermentor containing 16 l water: 80 min.

Artificial Seawater

Iron citrate	2 g (powder)
NaCl	389 g
$MgCl_2 \cdot 6H_2O$	176 g
Na_2SO_4	68.8 g
$CaCl_2$	36.0 g
Na_2HPO_4	0.16 g
SiO_2	0.30 g
Trace element stock soln.	20 ml
Stock soln.	200 ml
tap water	add 20 l

Trace element stock solution

H_3BO_3	0.611 g
$MnCl_2$	0.389 g
$CuSO_4$	0.056 g
$ZnSO_4 \cdot 7\ H_2O$	0.056 g
$Al_2\ (SO_4)_3 \cdot 18\ H_2O$	0.056 g
$NiSO_4 \cdot 6\ H_2O$	0.056 g
$CO\ (NO_3)_3 \cdot 6\ H_2O$	0.056 g
TiO_2	0.056 g
$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	0.056 g
LiCl	0.028 g
$SnCl_2$	0.028 g
KI	0.028 g
tap water	add 1 l

Stock solution

KCl	110 g
$NaHCO_3$	32 g
KBr	16 g
$SrCl_2 \cdot 6H_2O$	6.8 g (dissolved separately)
H_3BO_3	4.4 g
NaF	0.48 g
NH_4NO_3	0.32 g
tap water	add 2 l

5.6 Nutrients

M_2 medium (without seawater)

Mmalt extract	10 g
Glucose	4 g
Yeast extract	4 g
tap water	add 1 l

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar

M_2^+ medium (M_2 medium with seawater)

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
Artificial sea water	500 ml
tap water	500 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

M_2 100% Seawater + $CaCO_3$

Malt extract	10 g
Glucose	4 g
Yeast extract	4 g
$CaCO_3$	0.5 g
Artificial sea water	1000 ml

The pH was adjusted to 7.3 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

$CaCl_2$ Medium

Malt extract	40 g
Glucose	5 g
$CaCl_2$	45 g
tap water	1000 ml

The pH was adjusted to 7.8 using g 2N NaOH. Solid medium was prepared by adding 18 g of agar.

Luria-Bertani Medium (LB)

Trypton	10 g
yeast extract	5 g
NaCl	10 g
tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

Soja-Mannitol Medium

soybean meal (defatted)	20 g
D (-)-mannitol	20 g
Tap water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH. Solid medium was prepared by adding 18 g of agar.

M Test Agar (for test organisms *Escherichia coli*, *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus*, *Mucor miehei* (Tü 284):

malt extract	10 g
yeast extract	4 g
Glucose	4 g
Agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Sabouraud-Agar

(for test organism *Candida albicans*)

Glucose	40 g
Bacto peptone	10 g
agar	20 g
Demineralised water	1000 ml

The pH was adjusted to 7.8 using 2N NaOH.

Nutritional solution A

Soybean meal (defatted)	30 g
Glycerol	30 g
$CaCO_3$	2 g
Artificial sea water	750 ml
demineralised water	250 ml

Nutritional solution B

Starch	10 g
NZ-amine	5 g
Soybean meal	2g
Yeast extract	5 g
KNO_3	3 g

Algal extract	2.5 ml
Artificial sea water	750 ml
demineralised water	250 ml

5.7 Stock Solutions and Media for Cultivation of Algae

Fe-EDTA

0.7 g of $FeSO_4 \cdot 7\ H_2O$ and 0.93 g EDTA (Titriplex III) are dissolved in 80 ml of demineralised water at 60 °C and then diluted to 100 ml.

Trace element Solution II:

Solution A:

$MnSO_4 \cdot H_2O$	16.9 mg
$Na_2MoO_4 \cdot 2H_2O$	13.0 mg
$Co\ (NO_3)_2 \cdot 6H_2O$	10.0 mg

Salts are dissolved in 10 ml of demineralised water.

Solution B:

$CuSO_4 \cdot 5H_2O$	5.0 mg
H_3BO_3	10.0 mg
$ZnSO_4 \cdot 7H_2O$	10.0 mg

Salts are dissolved each in 10 ml of demineralised water. Solutions A is added to B and diluted to 100 ml with demineralised water.

Bold's Basal medium (BBM): (for algae *Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*.

$NaNO_3$	0.250 g
KH_2PO_4	0.175 g
K_2HPO_4	0.075 g
$MgSO_4 \cdot 7H_2O$	0.075 g
NaCl	0.025 g

$CaCl_2 \cdot 2H_2O$	0.025 g
Fe-EDTA	1.0 ml
trace element solution II	0.1 ml

Salts are dissolved in 10 ml of demineralised water and added to Fe-EDTA and trace element solution II. The mixture made to one litre with demineralised water. Solid medium was prepared by adding 18 g of agar.

5.8 Microbiological and Analytical Methods

5.8.1 Storage of Strains

All bacteria strains were stored in liquid nitrogen for long time. The strains were used to inoculate agar plates with the suitable media at room temperature.

5.8.2 Pre-Screening

The microbial isolates (obtained from culture collections) were cultured in a 1 l scale in 1 l-Erlenmeyer flasks each containing 200~250 ml of M_2 or (for marine strains) M_2^+ medium. The flasks were shaken for 3-5 days at 28 °C after, which the entire fermentation broth was freeze-dried and the residue extracted with ethyl acetate. The extracts were evaporated to dryness and used for the antimicrobial tests at 40 μg/paper disk.

5.8.3 Biological Screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (at concentration of ~10 mg/ml); 40 μl (= 400 μg extract) of this solution were dropped on paper disks by means of a Eppendorff pipette, dried under sterile conditions (flow box) and put on agar plates inoculated with the Gram-positive bacteria *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus* and *Streptomyces viridochromogenes* (Tü 57), the Gram-negative *Escherichia coli*; the yeast, *Candida albicans*; and the fungi, *Mucor miehei (*Tü 284) along with the three microalgae; *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*.

The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under daylight for micro-algae (96 hours). The diameters of the inhibition zones were measured by ruler.

5.8.4 Chemical and Pharmacological Screening

Samples of the extracts were separated on silica gel glass plates (10 × 20 cm) with two solvent systems $CHCl_3$/5% MeOH and $CHCl_3$/10% MeOH. After drying, the plates were photographed under UV light at 254 nm and marked at 366 nm, and subsequently stained by anisaldehyde and Ehrlich's reagent. Finally, the plates were scanned for documentation. For the pharmacological investigations, approximately 25 mg of the crude extract was sent to industrial partners.

5.8.5 Brine shrimp Microwell Cytotoxicity Assay

To a 500 ml separating funnel, filled with 400 ml of artificial sea water, 1 g of dried eggs of *Artemia salina L.* was added. The suspension was aerated by bubbling air into the funnel and kept for 24 to 48 hours at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. 30 to 40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 ml of salt water and the dead larvae counted (number N). A solution of 20 mg of the crude extract in 5 to 10 l DMSO was added and the plate kept at r.t. in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of *ca*. 0.5 ml methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in %. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 mg/test actinomycin D. The mortality rate M was calculated using the following formula:

$$M = \left[\frac{(A - B - N)}{(G - N)} \right] \cdot 100 \qquad \text{With}$$

M = percent of the dead larvae after 24 h.

A = number of the dead larvae after 24 h.

B = average number of the dead larvae in the blind samples after 24 h

N = number of the dead larvae before starting of the test.

G = total number of brine shrimps

The mortality rate with actinomycin must be 100% .

5.9 Primary Screening Results

5.9.1 Bases of evaluation

Antibiotic screening (disk diffusion test): The test is performed using paper discs with a diameter of 8 mm under standardized conditions (see above). If the inhibition zone is ranging from 11 to 20 mm, the compound is considered to be weakly active (+), from 21 to 30 mm designated as active (++) and over 30 mm is highly active (+++). - **Chemical screening:** evaluation of the separated bands by the number, intensity and colour reactions with different staining reagents on TLC. - **Toxicity test:** By counting survivors after 24 hrs, the mortality of the extracts was calculated (see above). The extracts, fractions or isolated compounds were considered inactive when the mortality rate was lower than 10% (-), from 10 to 59% as weakly active (+), from 60 to 95% as active (++) and over 95% as strongly active (+++).

6 Metabolites from Selected Strains

6.1 Terrestrial *Streptomyces* sp. ANK 264

6.1.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and weak against Artemia *salina* (brine shrimp).

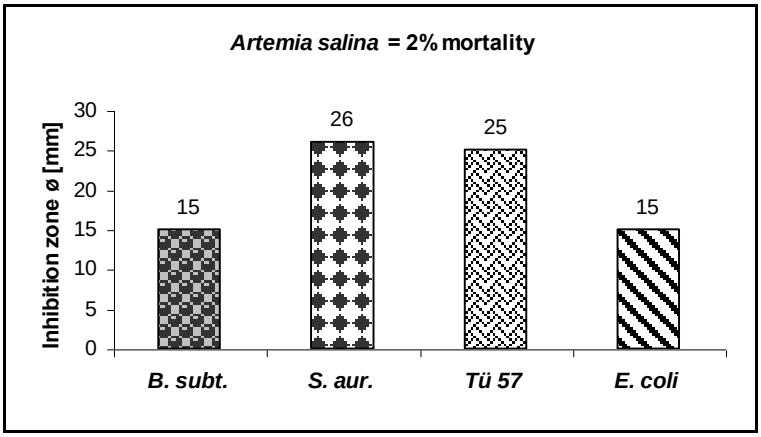


Figure 248: Biological activity of the crude extract from the terrestrial *Streptomyces* sp. ANK 264 at 40 μg/paper disk

6.1.2 Fermentation and working up

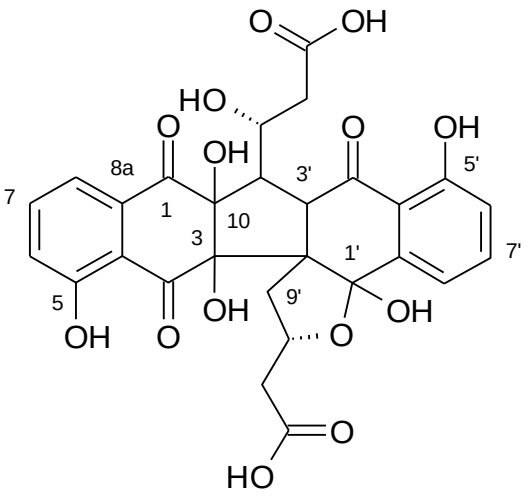
A well-grown sub-culture of the terrestrial *Streptomyces* sp. ANK 264 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (7.8 g).

6.1.3 Scale up and isolation

The crude extract 7.8 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pres-

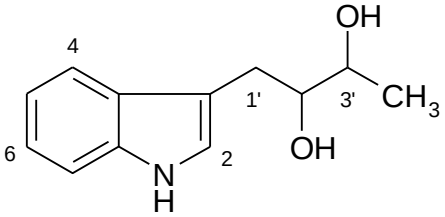
sure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). During elution with dichloromethane/methanol a white solid of deferrioxamine E (**41**) was precipitated. Three fractions were selected for further investigation based on the spot pattern on TLC. Fraction II was subjected to Sephadex LH-20 followed by RP-18 using MeOH/H_2O gradient (10 to 30 % MeOH) to deliver suhagcine I (**44**), and metabolite VIIb; fraction III was purified on Sephadex LH-20 using MeOH to afford pentenomycin I, 5-hydroxy-3-(1-hydroxy-2-metoxypropyl)-4-methyl-2(5H)furanone (**43**) and 2,5-furandimethanol (**46**). Fraction FIV was purified on Sephadex LH-20 using MeOH to afford juglorescein (**38**)**,** see Figure 4.

Juglorescein (38):



Oily substance, UV absorbing, black colour with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.20 (CH_2Cl_2/ 5% MeOH). − **^{1}H NMR**, **^{13}C NMR** (DMSO-d_6, 300, 125 MHz) see Table 4. − **^{1}H,^{1}H COSY** see Figure 7, **HMBC** see Figure 9. − (+)-**ESIMS:** *m*/*z* = 607 ($[M+Na]^+$, 1192.2 ($[2M+Na]^+$. − (+)-**ESIHRMS:** *m*/*z* = 607.10549 (calcd. 585.12388 For $[M+H]^+$), 607.10549 (calcd. 607.10582 For $[M+Na]^+$)

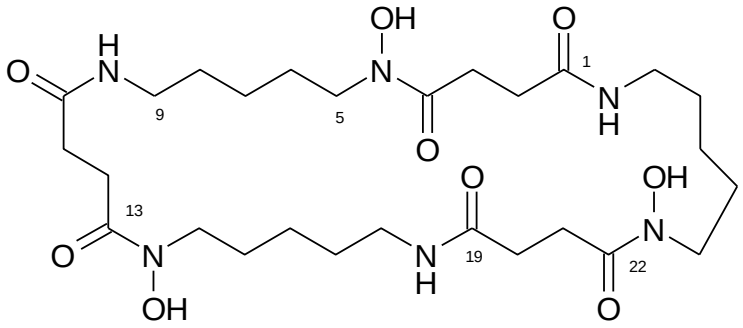
(**1*H*-indol-3-yl**)**-butane-2,3-diol** (**40**)**:**



Colourless oil substance, blue under UV, turned to red by spraying with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/7% MeOH). − **^{1}H NMR** (CD_3OD,

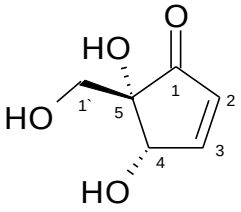
300 MHz): δ 7.55 (dd, J = 7.8, J = 1.9 Hz, 1H, H-7), 7.30 (dd, J = 8.0, J = 0.9 Hz, 1H, H-4), 7.09 (s, 1H, H-2), 7.06 (dt, J = 8.3, J = 1.0 Hz, 1H, H-6), 6.97 (dt, J = 10.3, J = 1.3 Hz, 1H, H-5), 3.68 (m, 2H, H-2', 3'), 3.03, 2.80 (ABX, J_{AB} = 14.5, J_{AX} = 4.9, J = 7.2 Hz, 2H, H-1'), 1.20 (d, J = 6.3, 3H, H_3-7). – (+)-**ESIMS:** m/z = 228 $[M+Na]^+$, 433 $[2M+Na]^+$. – (-)-**ESIMS:** m/z = 204 $[M-H]^-$, 409 $[2M-H]^-$.

Deferrioxamine E (41):



Colourless solid, UV inactive, turned to pale violet with anisalde-hyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.30 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 9.26 (s br, 3H, OH), 7.38 (s br, 3H, NH), 3.50 (t, J = 6.7 Hz, 6H, CH_2-5, 14, 23), 3.05 (q, J = 6.3, 6H, CH_2-9, 18, 27), 2.61 (t, J = 7.1 Hz, 6H, CH_2- 2, 11, 20) 2.33 (t, J = 7.0 Hz, 6H, CH_2-3, 12, 2), 1.55 (m, 6H, CH_2-6, 15, 24), 1.42 (m, 6H, CH_2-8, 17, 26), 1.26 (m, 6H, CH_2-7, 16, 25). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 171.1 (CO-1, 4, 10, 13, 19, 22), 47.1 (CH_2-5, 14, 23), 38.0 (CH_2-9, 18, 27), 30.1 (CH_2-3, 12, 21), 28.1 (CH_2-8, 17, 26), 27.2 (CH_2-2, 11, 22), 25.5 (CH_2-6, 15, 24), 22.9 (CH_2-7, 16, 25). – (+)-**HRESIMS:** m/z = 601.3555 (calcd. 601.3556 for $C_{27}H_{49}N_6O_9$), 623.3374 (calcd. 623.3375 for $C_{27}H_{48}N_6O_9Na$).

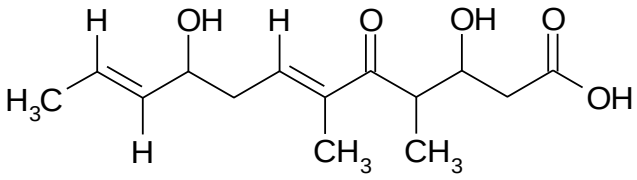
Pentenomycin I (42):



Colourless solid, UV active, change to blue with anisalde-hyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 28 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.64 (dd, J = 6.1, J = 2.4 Hz, 1H, H-3), 6.25 (dd, J = 6.1, J = 1.4 Hz, 1H,

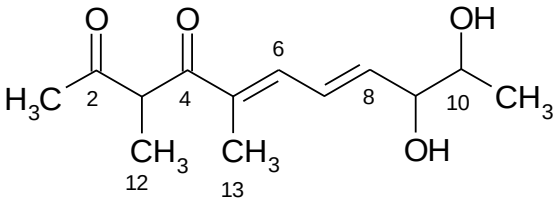
H-2), 4.75 (dd, *J* = 2.5, *J* = 1.4 Hz, H-4), 3.68 (d, *J* = 10.8 Hz, 1H, Ha-1'), 3.55 (d, *J* = 10.8 Hz, 1H, Hb-1'). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 208.7 (C_q-1) 164.2 (CH-3), 134.3 (CH-2), 76.3 (CH-4), 73.2 (C_q-5), 64.4 (CH_2-1').

Suhagcine I (44):



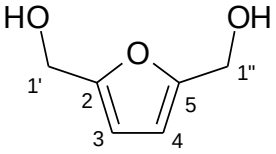
Colourless oil, turned to blue colour with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/5% MeOH). – $[\alpha]_D^{20}$ -11.39. – **^{1}H**, **^{13}C NMR** (CD_3OD, 300, 125 MHz) see Table 5. – **^{1}H,^{1}H COSY** and **HMBC** see Figure 25. – (-)-**ESIMS:** *m*/*z* = 269 $[M-H]^-$, 539 $[2M-H]^-$. – (+)-**HRESIMS:** *m*/*z* = 293.13608 (calcd for $C_{14}H_{22}O_5Na$, 293.13594).

Suhagcine II (45):



Oil colourless, blue colouration with anisaldehyde/sulphuric acid spraying reagent and heating. – $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz) and **^{13}C NMR** (CD_3OD, 125 MHz) see Table 6, **HMBC** and **^{1}H,^{1}H COSY** see Figure 32. – (+)-**ESIMS:** *m*/*z* = 263.1 $[M+Na]^+$, 503.2 $[2M+Na]^+$. – (+)-**HRESIMS:** *m*/*z* = 263.1255 (calcd. 263.1254 for $C_{13}H_{20}O_4Na$).

2,5-Furandimethanol (46):



Colourless oily substance, UV active turned to brownish colour with anisaldehyde. – $\boldsymbol{R_f}$ = 0.28 (CH_2Cl_2/7% MeOH). – **EI MS** (70 eV): *m*/*z* = (%) 128 ([M]•+, 24). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.22 (s, 2H, H-3, 4), 4.47 (s, 4H, H_2-1', 1'').

6.2 Terrestrial *Streptomyces* sp. ANK 251

6.2.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus* and *Artemia salina.*

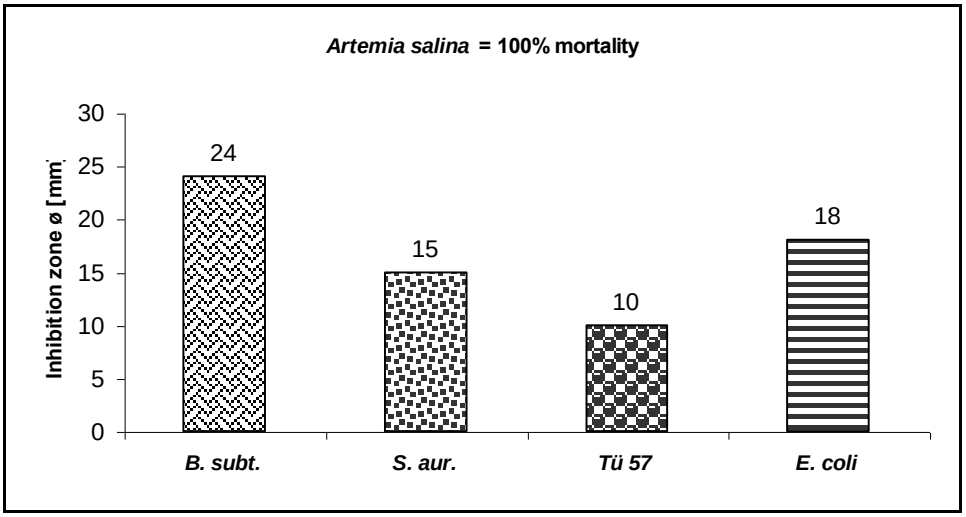


Figure 249: Biological activity of the crude extract from the terrestrial *Streptomyces* sp. ANK 251 at 40 μg/paper disk

6.2.2 Fermentation and working up

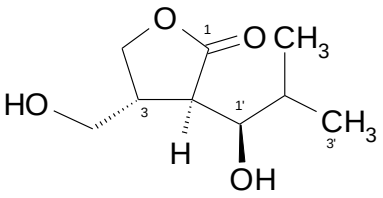
A well-grown sub-culture of the terrestrial *Streptomyces* sp. ANK 251 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (5.5 g).

6.2.3 Scale up and isolation

The crude extract 5.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 3 g of silica gel were added and this mixture was brought to dryness under reduced pres-

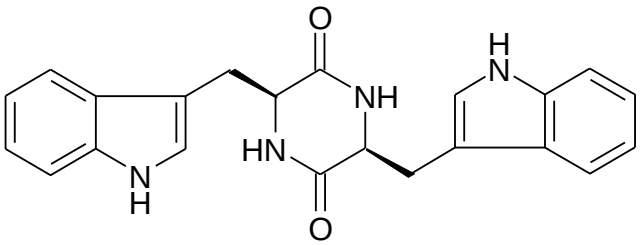
sure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). Under TLC control; three fractions were selected for further investigation. Fraction II subjected to Sephadex LH-20 to deliver virginiae butanolide F (**47**), fraction III was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O gradient (10 to 30 % MeOH) to afford 5'-methoxyinosine (**48**) and 5'-methoxyguanosine (**49**). Fraction IV was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O to afford fellutanine A (**50**); see Figure 34.

Virginiae butanolide F (47) :



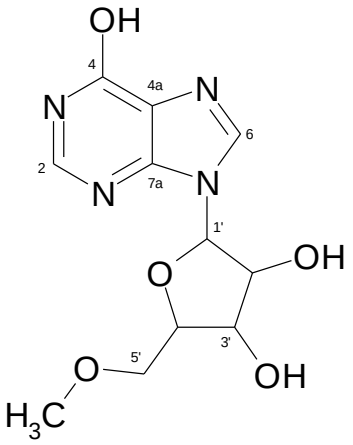
Colourless oil, 5.78 mg, UV absorbing, blue with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/10% MeOH). – **^{13}C** and **^{1}H NMR** (125, 300 MHz) in CD_3OD see Table 7. – **^{1}H,^{1}H COSY** and **HMBC** see Figure 38. – (+)-**ESIMS:** *m/z* = 211 $[M+Na]^+$, 399 $[2M+Na]^+$. – (-)-**ESIMS:** *m/z* = 187 $[M-H]^-$, 375 $[2M-H]^-$. – (+)-**HRESIMS:** *m/z* = 211.0950 $[M+Na]^+$ (calcd. 211.0941 for $C_9H_{16}NaO_4$).

Fellutanine A (50) :



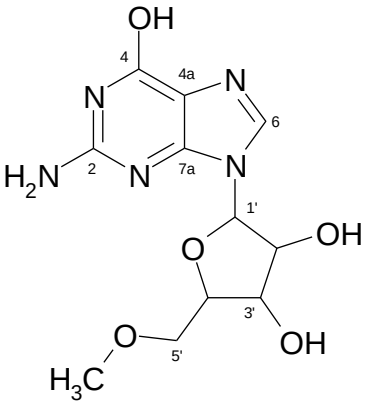
Yellow oil, 2.51 mg, UV absorbing, pink with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.23 (CH_2Cl_2/10% MeOH). – **^{13}C** and **^{1}H NMR** (CD_3OD, 125, 300 MHz) see Table 10. – **^{1}H,^{1}H COSY** and **HMBC** see Figure 52. – **(+)-ESIMS:** *m/z* = 395$[M+Na]^+$, 767$[2M+Na]^+$. – (-)-**ESIMS:** *m/z* = 371$[M-H]^-$, 743$[2M-H]^-$

5'-Methoxyinosine (48)



Colourless middle polar solid, UV absorbing at 254 nm, 4.0 mg, blue-green by spraying with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.35 ($CHCl_3$/MeOH 90: 10). – **^{13}C** and **^{1}H NMR** shifts (MeOH, 125, 300 MHz) of see Table 8. – **^{1}H,^{1}H COSY** and **HMBC** see Figure 43. – (+)-**ESIMS** *m/z* 305 $[M+Na]^+$, 587 $[2M+Na]^+$. – (-)-**ESIMS:** *m/z* = 281 $[M-H]^-$, 563 $[2M-H]^-$. – (+)-**HRESIMS:** *m/z* = 305.08578 $[M+Na]^+$ (calcd. 305.08564 for $C_{11}H_{15}N_5NaO_5$)**,** 283.10378 $[M+H]^+$ (calcd. 283.10370 for $C_{11}H_{15}N_4O_5$).

5'-Methoxyguanosine (49):



Colourless middle polar solid (2.5 mg), UV absorbing at 254 nm, turned to yellowish brown by spraying with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.25 ($CHCl_3$/MeOH 90: 10). – **^{13}C** and **^{1}H NMR** (DMSO-d_6, 125, 300 MHz, 100 °C) see Table 9. **^{1}H,^{1}H COSY** and **HMBC** see Figure 48 – (+)-**ESIMS:** *m/z* = 320 $[M+Na]^+$, 617 $[2M+Na]^+$. – (-)-**ESIMS:** *m/z* = 296 $[M-H]^-$, 593 $[2M-H]^-$. − **(+)-HRESIMS:** *m/z* = 320.09654 $[M+Na]^+$ (calcd. 320.09654 for $C_{11}H_{15}N_5NaO_5$). – (+)-**HRESIMS:** *m/z* = 298.11463 $[M+H]^+$ (calcd. 298.11460 for $C_{11}H_{16}N_5O_5$).

6.3 Terrestrial *Streptomyces* sp. ANK 275

6.3.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, *Mucor miehei* and *Artemia salina*

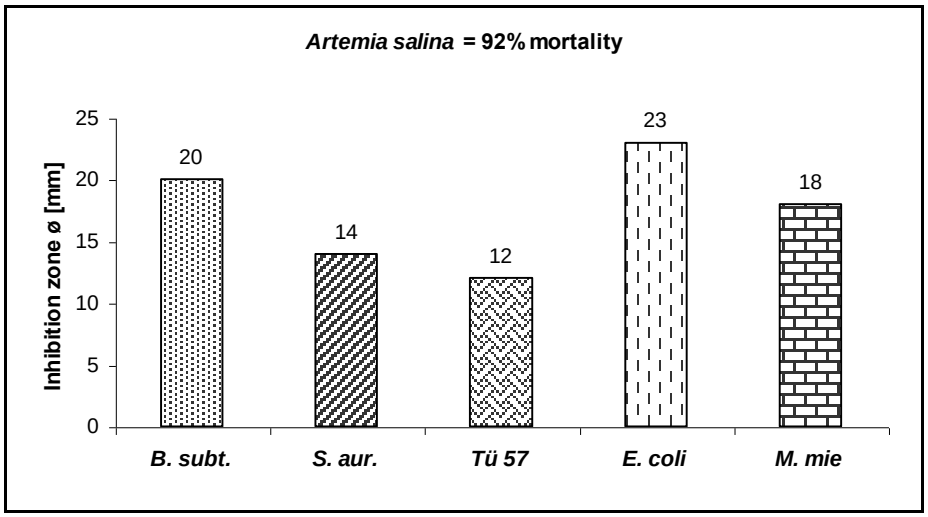


Figure 250: Biological activity of the crude extract from the terrestrial *Streptomyces* sp. ANK 275 at 40 μg/paper disk

6.3.2 Fermentation and working up

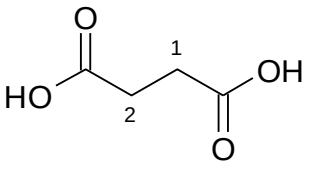
A well-grown sub-culture of the Terrestrial *Streptomyces* sp. ANK 275 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a black culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (7.2 g).

6.3.3 Scale up and isolation

The crude extract (7.2 g.) was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). Three fractions were selected for further investigation. Fraction II subjected to Sephadex LH-20 followed by RP-18 using MeOH/H_2O gradient (10 to 30 % MeOH) to deliver succinic acid (**51**) and

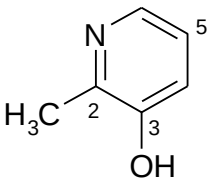
2-methylpyridine-3-ol (**55**), Fraction III was purified on Sephadex LH-20 using MeOH to afford N-(6-hydroxy-6-methyl-heptyl)-acetamide (**56**) and vanillic acid (**57**) . Fraction FIV was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O gradient (10 to 30 % MeOH) to afford 5'-acetoxy uridine (**58**) and 5'-acetoxy-2'-deoxy-thymidine (**59**); see Figure 55**.**

Succinic acid (51):



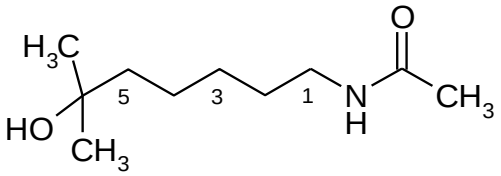
Colourless solid substance, 5.4 mg, UV inactive, colourless with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 2.55 (s, 4H, H_2-1, 2). − (+)-**ESIMS:** *m*/*z* = 141 $[M+Na]^+$, 259 $[2M+Na]^+$. − (-)-**ESIMS:** *m*/*z* = 117 $[M-H]^-$, 235 $[2M-H]^-$. − (+)-**HRESIMS:** *m*/*z* = 141.0163 $[M+Na]^+$, (calcd for $C_4H_6NaO_4$, 141.0158).

2-Methylpyridin-3-ol (55)



Colourless solid, 1.9 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.87 (d, *J* = 4.7 Hz, 1H, H-6), 7.20 (d, *J* = 8.2 Hz, 1H, H-4), 7.14 (dd, *J* = 8.1, *J* = 4.8 Hz, 1H, H-5), 2.41 (s, 3H, 2-CH_3). − **^{13}C NMR** (CD_3OD, 125 MHz): δ 154.1 (C_q-2), 147.3 (C_q-3), 138.6 (CH-6), 124.0 (CH-4,5), 18.0 (2-CH_3). − (+)-**ESIMS:** *m*/*z* = 110 ($[M+H]^+$, 25), 219 ($[2M+H]^+$, 2). − (+)-**HRESIMS:** *m*/*z* = 110.0602 $[M+H]^+$, (cald. for C_6H_8NO, 110.0600).

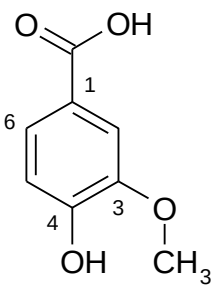
N-(6-Hydroxy-6-methyl-heptyl)-acetamide (56):



Colourless oil. − $\boldsymbol{R_f}$ = 0.53 (CH_2Cl_2/5% MeOH), UV inactive, green colouration with anisaldehyde /sulphuric acid. − **^{1}H NMR** (CD_3OD, 300 MHz): δ 3.14 (t, *J* = 7.0, 2H, H-1), 1.91 (s, 3H, $COCH_3$), 1.6- 1.2 (m, 8H, H_2-3, 4, 5, 6), 1.16 (s, 6H, 2

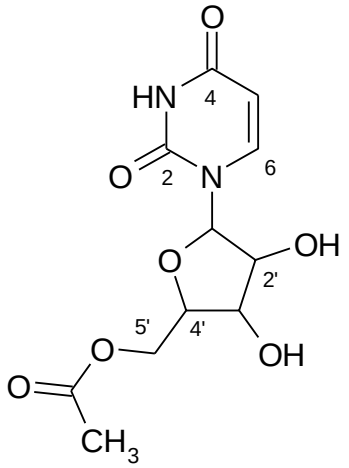
(CH_3)-6). − **^{13}C NMR** (CD_3OD, 125 MHz): δ 173.2 (CO), 71.4 (C_q-6), 44.7 (CH_2-5), 40.5 (CH_2-1), 30.4 (CH_2-5) 29.2 [2 (CH_3)-6], 30.4 (CH_2-4), 28.6 (CH_2-3), 25.1 (CH_2-2), 22.5 ($COCH_3$).

Vanillic acid (57) :



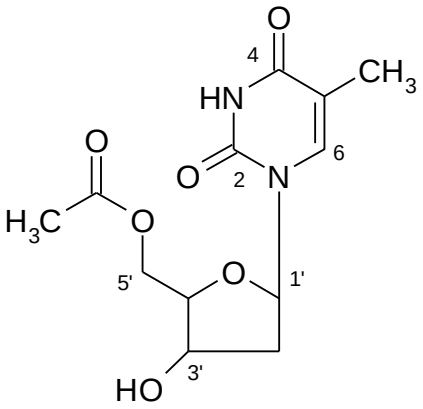
Colourless solid, 4.5 mg, strong UV absorbing at 254 nm, no colour reaction with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.29 (CH_2Cl_2/5% MeOH). − **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 7.49 (d, J = 1.56 Hz, 1H, H-2), 7.34 (dd, J = 8.19, J = 1.76 Hz, 1H, H-6), 6.69 (d , J = 8.10 Hz, 1H, H-5), 3.75 (s, 3H, OCH_3-3). − **EIMS** (70 eV, %)**:** m/z = 168 ($[M]^{+\cdot}$, 90), 153 ($[M-CH_4]^{+\cdot}$, 80), 109 $[M-CO_2H]^{+\cdot}$, 60).

5'-Acetyluridine (58):



Colourless solid, UV active compound turned to yellowish brown with anisaldehyde reagent and heating. − $\boldsymbol{R_f}$ = 0.14 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.68 (d, J = 8.1 Hz, 1H, H-6), 5.82 (d, J = 4.0 Hz, 1H, H-1'), 5.72 (d, J = 8.1 Hz, 1H, H-5), 4.31 (d, J = 4.0 Hz, 2H, H-5'), 4.18 (m, 1H, H-2'), 4.10 (m, 1H, H-4'), 4.08 (m, 1H, H-3'), 2.08 (s, 3H, 5'-OAc). − **^{13}C NMR** (CD_3OD, 125 MHz): δ 172.3 (5'-OAc), 166.8 (CO-4), 152.8 (CO-2), 142.0 (CH-6), 102.9 (CH-5), 91.8 (CH-1'), 82.9 (CH-4'), 75.2 (CH-2'), 71.3 (CH-3'), 64.7 (CH_2-5'), 20.7 (CH_3CO-5'). − (+)−**ESI:** m/z = 309 $[M+Na]^+$, 267 [$(M-COCH_3) + H]^+$, 511 [$(2M-COCH_3) + H]^+$.

5'-Acetyl-2'-deoxy-thymidine (59):



Colourless oil, 1.2 mg, UV absorbing, turned blue with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.10 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 150 MHz): δ 7.48 (s, 1H, H-6), 6.24 (t, J = 6.8 Hz, 1H, H-1'), 4.34 (m, 1H, H-3'), 4.32, 4.25 (2 m, 2H, H_2-5'), 4.05 (m, 1H, H-4'), 2.24 (m, 2H, H-2'), 2.08 (s, 3H, 5'-OAc), 1.89 (s, 3H, 5-CH_3). − **^{13}C NMR** (CD_3OD, 125 MHz): δ 172.3 (CO-5'), 166.3 (CO-4), 152.2 (CO-2), 137.5 (CH-6), 111.7 (C_q-5), 86.5 (CH-1'), 85.8 (CH-4'), 72.3 (CH-3'), 65.1 (CH_2-5'), 40.6 (CH-2'), 20.7 (CH_3CO-5'), 12.5 (CH_3-5). − (+)−**ESIMS:** m/z = 307 $[M+Na]^+$, 591 $[2M+Na]^+$. − (-)-**ESI:** m/z = 283 $[M-H]^-$, 567 $[2M-H]^-$. − (+)-**HRESIMS:** m/z = 307.0910 $[M+Na]^+$ (calcd for $C_{12}H_{16}N_2NaO_6$, 307.0901). − (-)-**HRESIMS:** m/z = 283.0938 $[M-H]^-$ (calcd for $C_{12}H_{15}N_2O_6$, 283.0936).

6.4 Terrestrial *Streptomyces* sp. Ank 329

6.4.1 Pre-screening

The crude extracts of Terrestrial *Streptomyces* sp. Ank 329 revealed strong biological activity against most test microorganisms

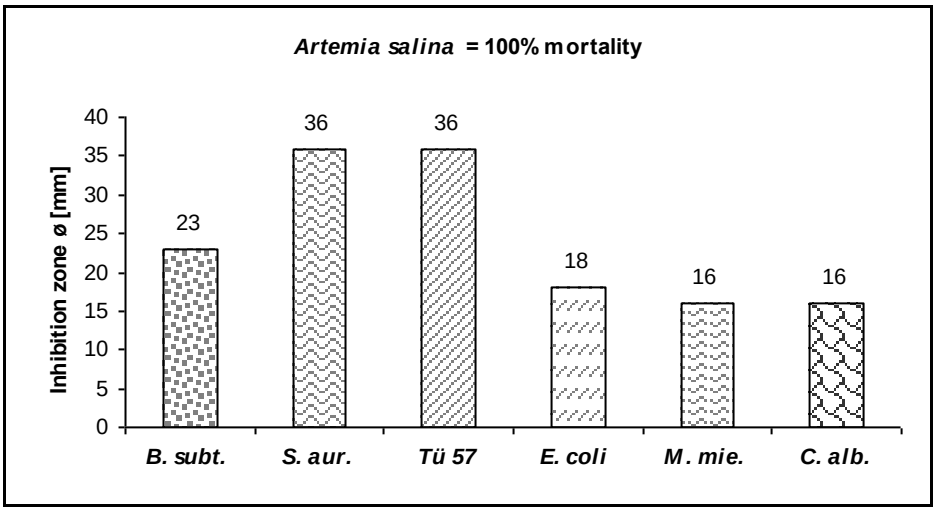


Figure 251: The biological activity for terrestrial *Streptomyces* sp. Ank 329 at 40 μg/paper disk

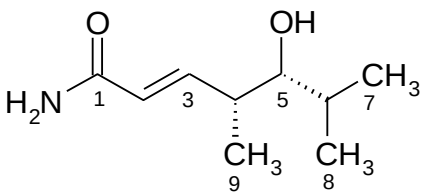
6.4.2 Fermentation and work-up

The strain *Streptomyces* sp. isolates Ank 329 formed red mycelial colonies. A 25-liter shaker culture of the terrestrial streptomycete strain Ank 329 was incubated a 28 °C using M_2^+ agar medium. The fermentor broth was harvested after 7 days, mixed with Celite, and then filtered. The filtrate and mycelia were subjected to extraction separately using XAD-16 for the water phase, followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times).

6.4.3 Scale up and isolation

The strain was scale up in to 25 l. The crude extract 3.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 2 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; four factions were selected for further investigation. Subfraction II afforded (*E*)-4-methyl-5-hydroxy-6-methyl-2-heptenamide (**60**), which showed UV absorbing bands at 254 nm and gave colour reaction with anisaldehyde/sulphuric acid reagent and also afforded 4-acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (**63**) which purified by RP-18 using MeOH/H_2O. Fraction III purified by Sephadex LH-20 eluted with methanol to give hydroxyl benzyl amine (**64**). Subfraction III purified by using RP-18 to produce 3-chloro-4-methoxybenzoic acid (**66**) and pyrrole-2-carboxamide (**67**). Fraction IV delivered indole-3-acetic acid (**65**); see Figure 75.

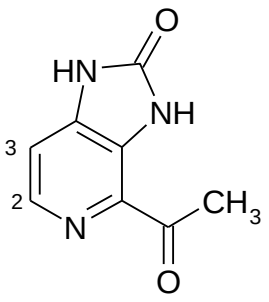
(*E*)-4-Methyl-5-hydroxy-6-methyl-2-heptenamide (60)



Colourless solid, 1.0 mg, slightly UV absorbing, turned to blue with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.23 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.72 (dd, ^3J = 15.5, ^4J = 8.5 Hz, 1H, H-3), 5.95 (d, *J*

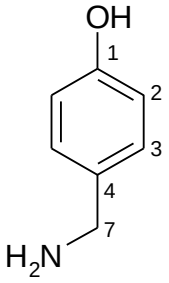
= 15.6 Hz, 1H, H-2), 3.16 (dd, *J* = 6.8, *J* = 5.1, 1H, H-5), 2.45 (hex, *J* = 6.2 Hz, 1H, H-4), 1.68 (m, 1H, H-6), 1.08 (d, *J* = 6.7, 3H, H-9), 0.90 (d, *J* = 6.8, 3H, H-7), 0.89 (d, *J* = 6.8, 3H, H-8) – **^{13}C NMR** (CD_3OD, 125 MHz): δ 170.9 (CO-1), 149.7 (CH-3), 123.5 (CH-2), 80.1 (CH-5), 41.4 (CH-4), 32.3 (CH-6), 20.4 (CH_3-7), 16.7 (CH_3-8), 15.5 (CH_3-9). **– ^{1}H,^{1}H COSY** and **HMBC** see Figure 79. **–** (+)-**ESIMS:** *m*/*z* = 194 $[M+Na]^+$. – (+)-**HRESIMS:** *m*/*z* = 194.1153 $[M+Na]^+$, (calcd 194.1151 for $C_9H_{17}NNaO_2$).

4-Acetyl-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (63):



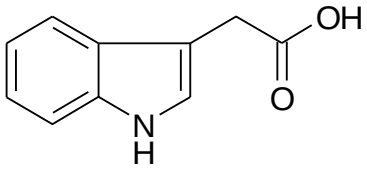
Colourless solid, 1.2 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.60 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.21 (d, *J* = 5.1 Hz, 1H, H-2), 7.20 (d, *J* = 5.1 Hz, 1H, H-3), 2.67 (s, 3H, $COCH_3$). − **^{13}C NMR** (CD_3OD, 125 MHz): δ 202.0 (CO-8), 159.3 (CO-2), 142.3 (CH-6), 139.1 (C_q-7a), 134.5 (C_q-4), 129.2 (C_q-3a), 109.2 (CH-7), 26.0 (CH_3-9). – (-)-**ESIMS:** *m*/*z* = 176 $[M-H]^-$. – (-)-**HRESIMS:** *m*/*z* = 176.04655 $[M-H]^-$, (calcd 176.04655 for $C_8H_7N_3O_2$).

4-Hydroxybenzyl amine (64):



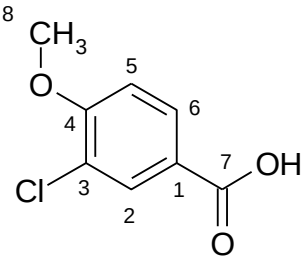
Colourless solid, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.30 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.09 (d, *J* = 8.6 Hz, 2H, H-3, 5), 6.71 (d, 2H, *J* = 8.6 Hz, H-2, 6), 3.38 (s, 2H, H-7). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 158.0 (C_q-1), 131.0 (CH-3, 5), 128.0 (C_q-4), 116.3 (CH-2, 6), 42.6 (CH-7).

Indole-3-acetic acid (65):



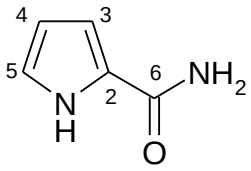
Colourless oil, 3.4 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.20 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.53 (d, J = 8.0 Hz, 1H, H-5), 7.37 (d, J = 8.2 Hz, 1H, H-4), 7.17 (s, 1H, H-2), 7.09 (t, J = 7.0, 1H, H-6), 7.01 (t, J = 7.0 Hz, 1H, H-7).

3-Chloro-4-methoxybenzoic acid (66):



Colourless solid, 1.8 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.26 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (MeOH, 300 MHz): δ 7.93 (d, J = 2.0 Hz, 1H, H-2), 7.85 (dd, J = 8.5, J = 2.1 Hz, 1H, H-6), 7.01 (d, J = 8.7 Hz, 1H, H-5), 3.89 (s, 3H, OCH_3-8). – **^{13}C NMR** (MeOH, 125 MHz): δ 173.6 (CO-7), 157.9 (C_q-4), 132.2 (CH-2), 130.3 (CH-6), 122.4 (C_q-1), 112.0 (CH-6), 56.6 (CH_3-8). − (-)-**ESIMS:** m/z = 185 $[M\text{-}H]^-$. – (-)-**HRESIMS:** m/z = 185.00107 $[M\text{-}H]^-$, (calcd 185.00109 for $C_8H_6O_3Cl$).

Pyrrole-2-carboxamide (67):



Colourless solid, 1.5 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.24 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.82 (d, J = 5.4 Hz, 1H, H-5), 6.73 (t, J = 4.6 Hz, 1H, H-4), 6.05 (d, J = 4.9 Hz, 1H, H-3). − **^{13} C NMR** (CD_3OD, 125 MHz), 162.0 (CO-6), 121.1 (CH-5), 110.4 (CH-4), 108.3 (CH-3), 126.1 (C_q-2). − **EIMS** (70 eV)**:** m/z = 110 ($[M]^{+\cdot}$, 100), 66 ($[C_4H_4N]^{+\cdot}$, 25).

6.5 Terrestrial *Streptomyces* sp. ANK 312

6.5.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Staphylococcus aureus.*

6.5.2 Fermentation and working up

A well-grown sub-culture of the terrestrial *Streptomyces* sp. ANK 312 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated *in vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (4.6 g).

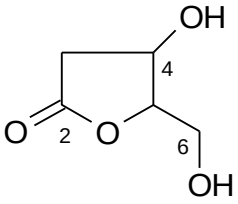
6.5.3 Scale up and isolation

The crude extract 4.6 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 3 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). Under TLC control, three fractions were selected for further investigation. Fraction II was subjected to Sephadex LH-20 to afford fatty acids, FIII delivered deoxyribonolactone (**68**) and N-(4,5-dimethyl-2-oxo-tetrahydrofuran-3-yl)-acetamide (**69**), FIV afforded adenosine and indole carboxylic acid; see Figure 88.

Deoxyribonolactone (68):

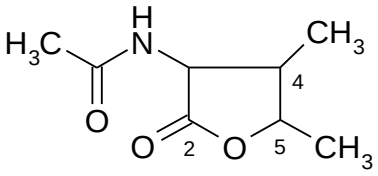
Brown oily substance, no UV absorbance at 254 nm or 362 nm, gave pink colour with anisaldehyde/sulphuric acid reagent and heating. − $\boldsymbol{R_f}$ = 1.2 (CH_2Cl_2/5%

CH_3OH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.42 (dt, J = 4.7, J = 2.3 Hz, 1H, H-4), 4.36 (dt, J = 3.5, J = 2.3 Hz, 1H, H-5), 3.76, 3.68 (ABX, J_{AB} = 12.4, J_{AX} = 3.3, J_{BX} = 3.3 Hz, 2H, H_2-6), 2.91, 2.37 (ABX, J_{AB} = 18.0, J_{AX} = 6.7, J_{BX} = 2.6 Hz, 2H, H_2-3). – **^{13}C NMR** (CD_3OD, 300 MHz): δ 178.6 (CO-2), 90.1 (CH-5), 69.6 (CH-4), 62.5 (CH_2-6), 39.1 (CH_2-3). – (+)-**ESIMS** *m/z* 155 $[M+Na]^+$, 287 $[2M+Na]^+$. – (+)-**HRESIMS:** *m*/*z* = 155.0325 (calcd. 155.0315 for $C_5H_8NaO_4$), 287.0755 (calcd. 287.0737 for $C_{10}H_{16}N_aO_8$).



Desmodilactone (69):

Colourless solid, UV absorbance in active, blue colour with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 1.2 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.65 (d, J = 8.8 Hz, 1H, H-3), 4.36 (m, 1H, H-5), 2.38 (m, 1H, H-4), 2.02 (s, 3H, 3-$NHCOCH_3$), 1.40 (d, J = 6.4, 3H, 5-H_3C), 0.96 (d, J = 7.1, 3H, 4-CH_3). – **^{13}C NMR**: δ 176.6 (CO-2), 173.3 (3-NHCO), 84.0 (CH-5), 53.2 (CH-3), 41.0 (CH-4), 22.1 (3-$NHCOCH_3$), 19.9 (CH_3-5), 12.7 (CH_3-4). – (+)-**ESIMS:** *m*/*z* = 194 $[M+Na]^+$, 364.8 $[2M+Na]^+$. – (+)-**HRESIMS:** *m*/*z* = 172.09683 (calcd. 172.09682 for $C_8H_{14}NO_3$), 194.07873 (calcd. 194.07876 for $C_8H_{13}NNaO_3$).



6.6 Terrestrial *Streptomyces* sp. ANK 320

6.6.1 Pre-screening

The crude extract of the terrestrial *Streptomyces* sp. ANK 320 showed weak biological activity against microorganisms (*Mucor miehei* and *Artemia salina*)

6.6.2 Fermentation and working up

A well-grown sub-culture of the Terrestrial *Streptomyces* sp. ANK 320 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the strain showed a brown cul-

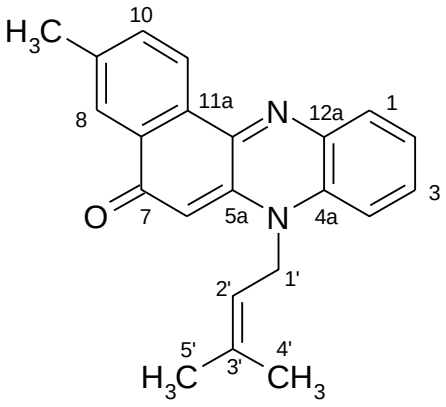
ture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (5.2 g).

6.6.3 Scale up and isolation

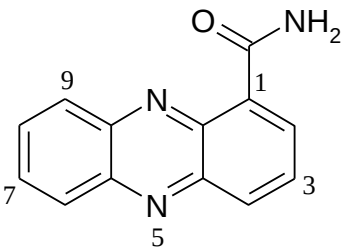
The crude extract 5.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 3 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). The first fraction contained fatty acids and was not invested. Fraction II was subjected to PTLC to deliver chromophenazine A (**74**) and phenazine-1-carboxyamide, in the same way FIII afforded α-picolinamid (**76**) from PTLC plates by using (CH_2Cl_2/10% CH_3OH), see Figure 100.

Chromophenazine A (74):

Orange solid, 1.0 mg, UV absorbing band at 254 nm, blue colour with spraying anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.36 (5% MeOH/ CH_2Cl_2). − **^{1}H, ^{13}C NMR** ($CDCl_3$, 300, 125 MHz) see Table 12. − (+)-**ESIMS:** *m*/*z* = 329 ($[M+H]^+$, − (+)-**HRESIMS:** *m*/*z* = 329.16498 $[M+H]^+$ (calcd for $C_{22}H_{21}N_2O$, 329.16484).

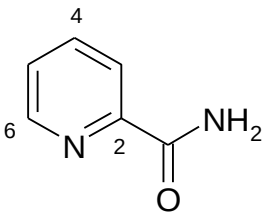


Phenazine-1-carboxamide (75):



Yellow needles, UV absorbing zone, turned to yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.29 (CH_2Cl_2/5% MeOH. – **^{1}H NMR** (300 MHz, CD_3OD): δ 8.83 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-2), 8.42 (dd, ^3J = 8.7, J = 1.5 Hz, 1H, H-4), 8.26 (m, 1H, H-6), 8.23 (m, 1H, H-9), 7.96 (dd, ^3J = 7.1, J = 1.5 Hz, 1H, H-3), 7.93 (dd, ^3J = 7.0 Hz, J = 1.6 Hz, 1H, H-7), 7.88 (dd, ^3J = 7.7 Hz, J = 1.0 Hz, 1H, H-8). $^-$ (+)-**ESIMS:** m/z = 246 ($[M+Na]^+$, 469 ($[2M+Na]^+$. – (+)-**HRESIMS:** m/z = 246.06383 $[M+Na]^+$ (calcd. 246.06378 for $C_{13}H_9N_3ONa$).

Picolinamid (76):



Yellow crystals, 1.5 mg, UV absorbing, turned to yellow with anisaldehyde/sulphuric acid spray reagent. − $\boldsymbol{R_f}$ = 0.30 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.62 (dd, J = 4.8, J = 2.5, 1H, H-6), 8.09 (td, J = 7.8, J = 2.1, 1H, H-3), 7.94 (td, J = 7.7, J = 1.7, 1H, H-4), 7.53 (dq, J = 7.6, J = 4.8, 1H, H-5). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 169.3 (2-CONH), 150.9 (C_q-2), 149.7 (CH-6), 138.6 (CH-4), 127.7 (CH-5), 123.1 (CH-3). – **EIMS** (70 eV): m/z (%) = 122 ([M]•+, 60), 79 ([M-$CONH_2$]•+, 100).

6.7 Terrestrial *Streptomyces* sp. ADM 9

6.7.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and special activity against Artemia *salina.*

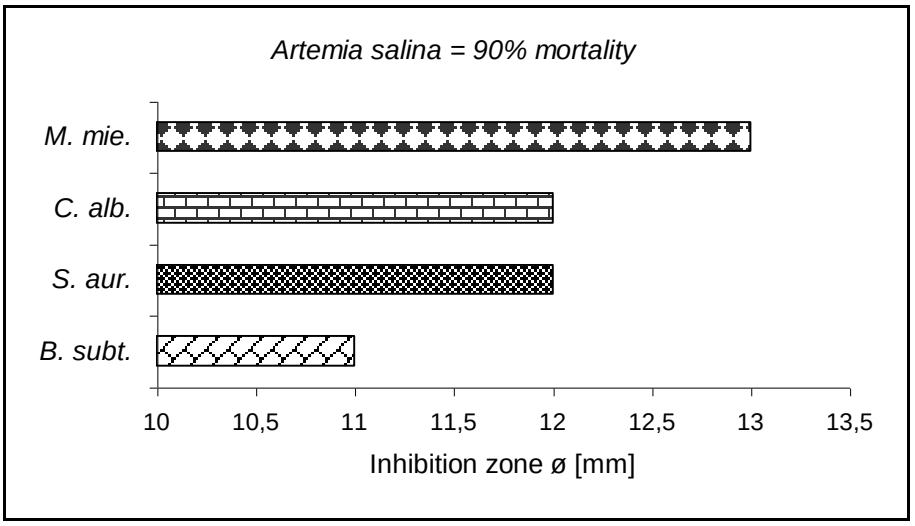


Figure 252: The activity of crude extracts from the terrestrial *Streptomyces* sp. ADM 9 at 40 μg/paper disk

6.7.2 Fermentation and working up

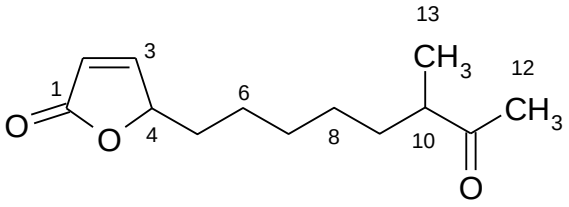
A well-grown sub-culture of the terrestrial *Streptomyces* sp. ADM 9 was used for inoculation of a 20 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the strain showed a red culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (3.5 g).

6.7.3 Scale up and isolation

The crude extract 3.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; three factions were selected for further investigation. The low polar compounds from fraction II, which showed no UV absorbing bands at 254 nm and gave violet to red colour reaction with anisalde-

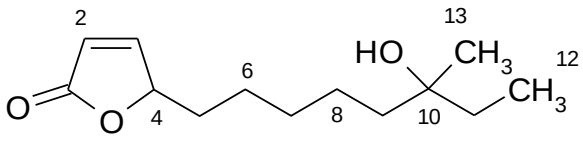
hyde/sulphuric acid, was purified to isolated 4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (**77**) (5.6 mg), 4,10-dihydroxy-10-methyl-dodec-2-en-1,4-olide (**78**) (6.1 mg) and tryptophol (7.3 mg). From fraction III was chromatographed on Sephadex LH-20 column to isolated 4-hydroxy benzoic acid and indole-3-carboxylic acid (7.1 mg). Moreover, fraction IV was chromatographed on Sephadex LH-20 column using MeOH and afforded 3-(hydroxyacetyl)indole (5.3 mg) and ferulic acid (**79**) (4.9 mg), see Figure 109.

4-Hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide (77):



Colourless oil, 5.6 mg. violet with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.38 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.70 (dd, J = 5.7, J = 1.5 Hz, 1H, H-3), 6.11 (dd, J = 5.7, J = 2.0 Hz, 1H, H-2), 5.12 (m, 1H, H-4), 2.56 (m, 1H, H-10), 2.13 (s, 3H, H-12), 1.70-1.20 (m, 10H, H-5, 6, 7, 8, 9), 1.06 (d, ^3J = 7.0 Hz, 3H, H-13). – (+)-**ESIM:** m/z = 470 $[2M+Na]^+$, 247 $[M+Na]^+$.

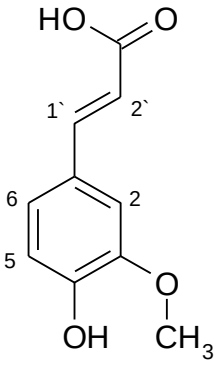
4,10-Dihydroxy-10-methyl-dodec-2-en-1,4-olide (78):



Colourless oil, 6.1 mg , violet with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.50 ($CHCl_3$/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.70 (dd, J = 5.5, J = 1.7 Hz, 1H, H-3), 6.11 (dd, J = 5.7, J = 2.0 Hz, 1H, H-2), 5.13 (m, 1H, H-4), 1.80 (m, 1H, H_a-5), 1.61 (m, 1H, Hb-5), 1.50-1.20 (m, 8H), 1.09 (s, 3H, H-13) 0.87 (t, J = 7.6 Hz, 3H, H-12). – **(+)-ESIMS:** m/z = 249 $[M+Na]^+$, 475 $[2M+Na]^+$.

Ferulic acid (79):

Colourless oil, (4.9 mg), UV absorption band at 254 nm, stained to violet with anisaldehyde/sulphuric acid and heating. – R_f = 0.3 (CH_2Cl_2/10% MeOH) – **^{1}H NMR** spectrum (CD_3OD, 300 MHz): δ 7.55 (d, J = 15.9 Hz, 1H, H-1'), 6.30 (d, J = 15.9 Hz, 1H, H-2'), 7.16 (d, J = 1.9 Hz, 1H, H-2), 7.04 (dd, J = 2.1 Hz, J = 8.2 Hz, 1H, H-6), 6.79 (d, J = 8.2 Hz, 1H, H-5).



6.8 Terrestrial *Streptomyces* sp. ANK 179

6.8.1 Pre-screening:

The crude extract showed in the agar diffusion test activity against different microorganisms *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and special activity against Artemia *salina.* The spraying with anisaldehyde/sulphuric acid and heating produced violet and yellow colour spots.

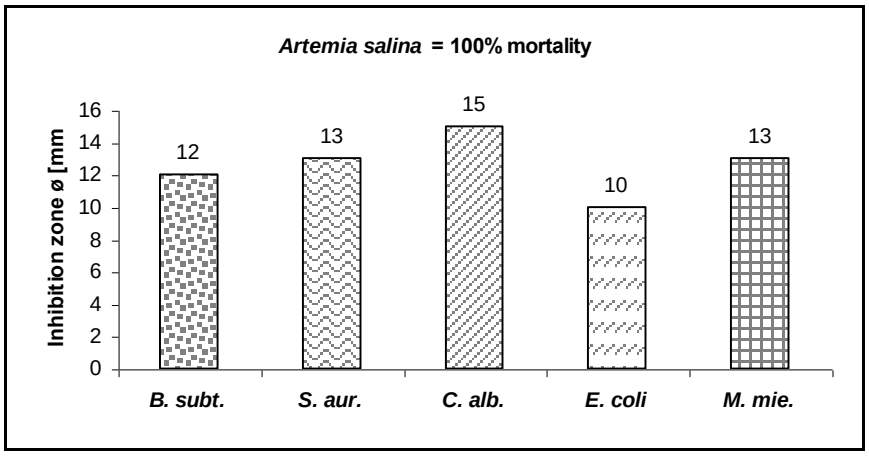


Figure 253: Biological activity for the crude extract of ANK 179 at 40 μg/paper disk

6.8.2 Fermentation and working up

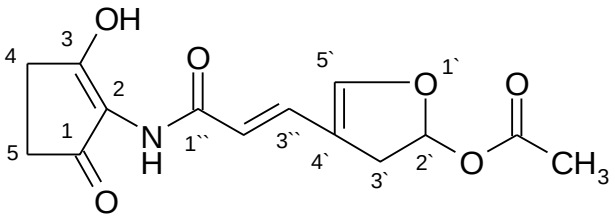
The strain *Streptomyces* sp. isolates Ank 179 formed brown mycelial colonies. A 20-liter shaker culture of the terrestrial streptomycete strain Ank 179 was to incubate at 28 °C using M_2^+ agar medium. The resulting red culture broth was harvested after 7 days, mixed with ca. 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the aqueous filtrate and a mycelial fraction. The water phase was sub-

jected to extraction separately using XAD-16 resin followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times) followed by acetone (3 times). The EtOAc and acetone phases were evaporated and dryness. On TLC the three crude extracts showed similar zones, accordingly they were collected together, the extract was defatted with cyclohexane by decantation to get 2.1 g of yellowish-brown crude extract.

6.8.3 Scale up and isolation

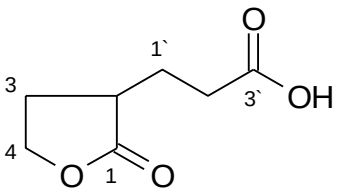
The crude extract subjected to silica gel column chromatography using CH_2Cl_2/MeOH gradient (column 3 x 60 cm, 0 to 20 % MeOH). During fractionation pale yellow needle-shaped delivered and elucidated as reductiomycin (**80**) after HNMR spectrum. Fraction C was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H2O gradient (10 to 30 % MeOH) to deliver 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid (**81**), see Figure 113.

Reductiomycin (80):



Pale yellow needle-shaped crystals, 50 mg, UV absorbing band at 254 nm, deep green colour by sparing with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.30 ($CHCl_3$/4% MeOH) − **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 13.91 (sbr, 1H, OH), 8.05 (sbr, 1H, NH), 7.49 (d, J = 15.0 Hz, 1H, H-2") and 5.9 (d, J = 15.0 Hz, 1H, H-3), 6.86 (s, 1H, H-5'), 6.73 (dd, J = 7.5, J = 2.3 Hz, 1H, H-2'), 3.03 (dd, J = 1.4, J = 7.5 Hz, 1H, H-3'a), 2.75- 2.50 (m, 5H, H-3'b, 4, 5), 2.12 (s, 3H, CH_3COO).

3-(2-Oxo-tetrahydrofuran-3-yl)-propionic acid (81):



Colourless oil, violet colouration with anisaldehyde/sulfuric acid spraying reagent. − $\boldsymbol{R_f}$ = 0.20 (CH_2Cl_2/3% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = 0° (1 mg/1 ml MeOH). **UV/V**IS: λ_{max} (log ε) (20 µg/ml MeOH): 203

(3.40), 201 (3.41); (MeOH/NaOH): 207 (3.33), 205 (3.35). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.35 (ddd, J = 17.5, J = 8.7, J = 2.5 Hz, 1H, H4-a), 4.20 (m, 1H, H4-b), 2.68 (m, 1H, H-2), 2.44 (t, J = 7.6, 1H, H-2'), 2.43 (m, 1H, H3-a), 1.96 (m, 1H, H3-b), 2.10 (m, 1H, H1'-a), 1.72 (m, 1H, H1'-b). – **^{13}C NMR** (CD_3OD, 500 MHz): δ 181.7 (CO-1), 177.0 (CO-3'), 68.2 (CH-4), 39.8) CH-2), 32.7 (CH_2-2'), 29.6 (CH_2-3), 26.8) CH_2-1') **– ^{1}H,^{1}H COSY** and **HMBC** see Figure 119. **– (-)-ESIMS:** m/z = 157.3 $[M-H]^-$. – (-)-**HRESIMS:** m/z = 157.05063 $[M-H]^-$ (calcd. 157.05063 for $C_7H_9O_4$).

6.9 Terrestrial *Streptomyces* sp. ANK 174

6.9.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and good activity against *Artemia salina.*

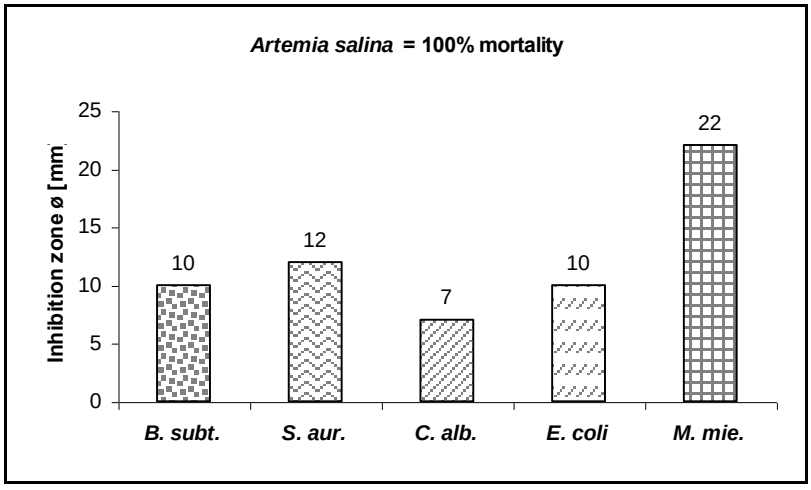


Figure 254: Biological activity of the crude extract from the terrestrial *Streptomyces* sp. ANK 174 at 40 μg/paper disk

6.9.2 Fermentation and working up

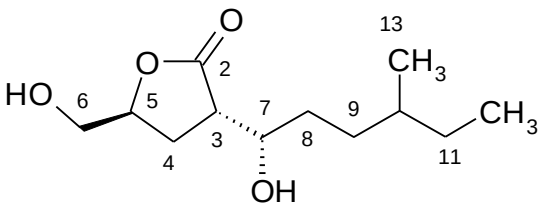
The strain *Streptomyces* sp. isolate Ank 174 formed brown mycelial colonies. A 20-liter shaker culture of the terrestrial streptomycete strain Ank 174 was incubating at 28 °C using M_2^+ agar medium. The resulting red culture broth was harvested after 7 days, mixed with ca. 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the aqueous filtrate and a mycelial fraction. The aqueous phase was

subjected to extraction separately using XAD-16 resin followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times) followed by acetone (3 times). The EtOAc and acetone phases were evaporated and dryness. On TLC the three crude extracts showed similar zones, accordingly they were collected together, the extract was defatted with cyclohexane by decantation to get 2.1 g of yellowish-brown crude extract.

6.9.3 Scale up and isolation

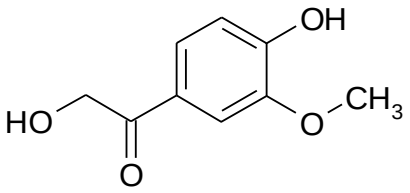
The crude extract subjected to silica gel column chromatography using CH_2Cl_2/MeOH gradient (column 3 x 60 cm, 0 to 20 % MeOH). Fraction B was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/H_2O gradient (10 to 30 % MeOH) to deliver hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (**84**) and 2-hydroxy-1-(3,4-dimethoxy-phenyl)-ethanone (**85**), respectively. Fraction C was purified on Sephadex LH-20 using MeOH to precipitate poly-(hydroxybutyric acid); Fraction D was subjected to Sephadex LH-20 followed by RP-18 using MeOH/H_2O gradient to afford lactone R4 (**82**), see Figure 120.

Lactone R4 (82):



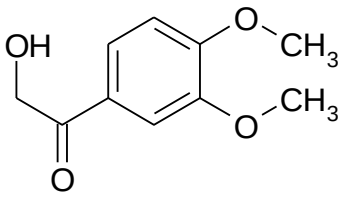
Colourless oil, 1.5 mg, UV absorbing, turned to blue with anisaldehyde/sulphuric acid spray reagent and heating. – $\boldsymbol{R_f}$ = 0.24 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.49 (m, 1H, H-5), 3.99 (m, 1H, H-7), 3.73 (dd, J = 12.3, J = 3.4, 1H, H-6a), 3.62 (dd, J = 12.3, J = 6.2 Hz, 1H, H-6b), 2.85 (m, 1H, H-3), 2.19 (m, 1H, H-4a), 2.04 (m, 1H, H-4b), 1.60- 1.30 (m, 5H, H-11, 10, 8a, 9a), 1.14 (m, 2H, H-9b, 8b), 0.88 (m, 6H, H_3-12, CH_3-13). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 180.1 (CO-2), 81.0 (CH-5), 70.4 (CH-7), 64.9 (CH_2-6), 47.8 (CH-3), 33.8 (CH-10), 35.7 (CH_2-8), 34.2 (CH_2-9), 30.5 (CH_2-11), 24.3 (CH_2-4), 19.6 (CH_3-13), 11.7 (CH_3-12). **^{1}H,^{1}H COSY** and **HMBC** see Figure 124.

Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (84):



Colourless solid, greenish-blue colouration with anisaldehyde/sulfuric acid spraying reagent. − $\boldsymbol{R_f}$ = 0.52 (CH_2Cl_2/3% MeOH). – **UV/VIS**: λ_{max} (log ε) (20 mg/ml MeOH): (MeOH) 301 (3.93), 276 (4.02), 229 (4.17), 204 (4.19); (MeOH/HCl): 302 (3.93), 276 (4.05), 229 (4.16), 204 (4.19); (MeOH/NaOH): 344 (4.32), 327 (4.80), 210. − **^{1}H, ^{13}C NMR** (300, 150 MHz, CD_3OD) see Table 13. – **HMBC** see Figure 128. – (+)-**ESIMS:** *m*/*z* = 385.2 $[2M-2H+Na]^+$, 181.2 $[M-H]^-$. − (+)-**ESIHRMS:** *m*/*z* = 183.06517 $[M+H]^+$ (calcd 183.06519 for $C_9H_{11}O_4$), 205.04711 $[M+Na]^+$ (calcd. 205.04714 for $C_9H_{10}O_4Na$). − (-)-**ESIHRMS:** *m*/*z* = 181.05057 $[M-H]^-$ (calcd. 181.05062 for $C_9H_9O_4$).

1-(3,4-Dimethoxy-phenyl)-2-hydroxy-ethanone (85):



Colourless solid, greenish-blue colouration with anisaldehyde/sulfuric acid reagent. − $\boldsymbol{R_f}$ = 0.64 (CH_2Cl_2/3% MeOH). − **UV/VIS**: λ_{max} (log ε) (20 mg/ml MeOH): (MeOH) 301 (3.55), 273 (3.68), 226 (3.89), 202 (3.94); (MeOH/HCl): 300 (3.50), 273 (3.64), 227 (3.85), 202 (3.89); (MeOH/NaOH): 304 (3.52), 273 (3.62), 210 (3.74), 207 (3.79) nm. − **^{1}H, ^{13}C NMR** (CD_3OD, 300, 150 MHz) see Table 14. − **EIMS** (70 eV, (%)**:** *m*/*z* = 196 ($[M]^{+\cdot}$ 18), 165 (100), 137 (8), 79 (12), 77 (10), 51 (10). − (+)-**EIHRMS:** *m*/*z* = $[M+Na]^+$ 219.06271 (calcd 219.06279 for $C_{10}H_{12}O_4Na$).

6.10 Terrestrial *Streptomyces* sp. WO 990

6.10.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, *Mucor miehei* and *Artemia salina*

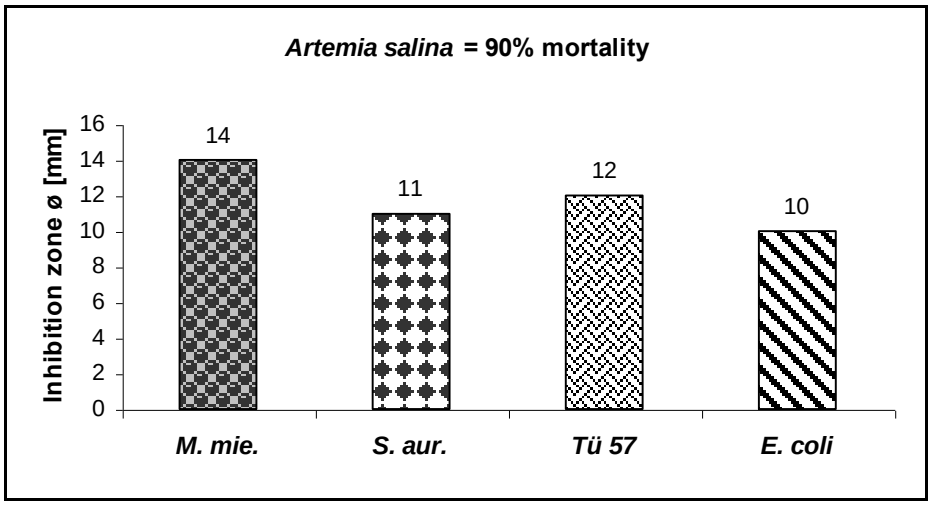


Figure 255: The biological activity of the terrestrial *Streptomyces* sp. Wo 990 at 40 μg/paper disk

6.10.2 Fermentation and working up

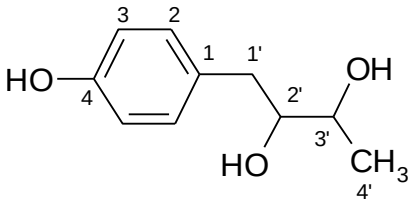
A well-grown sub-culture of the terrestrial *Streptomyces* sp. WO 990 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before sterilisation. After 7 days cultivation at 28 °C, the strain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (5.5 g).

6.10.3 Scale up and isolation

The crude extract 5.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 3 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). The first fraction not invested due to contained highly fatty acids, Fraction II subjected to silica gel (CH_2Cl_2/MeOH) gradient to deliver Indolyl-3-glyoxylamide (**93**), indole-3-carbaldehyde (**97**) and 4-hydroxybenzaldehyd (**96**), Fraction III was purified on Sephadex LH-20 using MeOH to afford 1-(4-

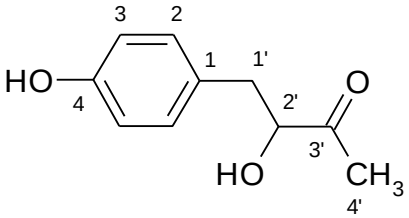
hydroxy-phenyl)-butane-2,3-diol (**86**), 3-hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (**89**) and N-Acetyl-2-aminophenol (**92**). Fraction FIV was purified on Sephadex LH-20 using MeOH followed by RP-18 using a H_2O/MeOH gradient to afford deoxyuridine (**94**) and thymine (**95**); see Figure 131.

1-(4-Hydroxy-phenyl)-butane-2,3-diol (86):



Colourless oil, 3.5 mg, UV absorbing, turned to red with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.22 (CH_2Cl_2/5% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = +5.0 (*c* 0.14, MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.05 (d, *J* = 8.5 Hz, 2H, H-2,6), 6.68 (d, *J* = 8.5 Hz, 2H, H-3,5), 3.61 (m, 1H, H-3'), 3.49 (m, 1H, H-2'), 2.76, 2.55 (ABX, J_{AB} = 13.9, J_{AX} = 4.7, J_{BX} = 8.5, Hz, 2H, H-1'), 1.16 (d, *J* = 6.4 Hz, 3H, H-4'). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 156.7 (C_q-4), 131.36 (CH-2,6), 131.32 (C_q-1), 116.0 (CH-3, 5), 77.8 (CH-2'), 70.4 (CH-3'), 39.4 (CH_2-1') 19.3 (CH_3-4'). **– ^{1}H,^{1}H COSY** and **HMBC** see Figure 136. **– (+)-ESIMS:** *m*/*z* = 205 $[M+Na]^+$, 387.2 $[2M+Na]^+$. – (-)-**ESIMS:** *m*/*z* = 181 $[M-H]^-$. – (+)-**HRESIMS:** *m*/*z* = 205.0837 $[M+Na]^+$ (calcd. 205.0835 for $C_{10}H_{14}NaO_3$). – (-)-**HRESIMS:** *m*/*z* = 181.0872 $[M-H]^-$ (calcd. 181.0870 for $C_{10}H_{13}O_3$).

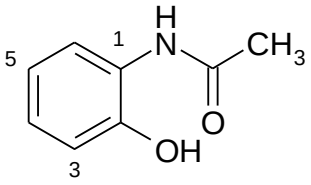
3-Hydroxy-4-(4-hydroxy-phenyl)-butan-2-one (89):



Colourless oil, 2.5 mg, UV absorbing, turned to red with anisaldehyde/sulphuric acid spray reagent. – $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/5% MeOH). – $\boldsymbol{[\alpha]_D^{20}}$ = +3.3 (*c* 0.18, MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.04 (d, *J* = 8.5 Hz, 2H, H-2,6), 6.68 (d, *J* = 8.5 Hz, 2H, H-3,5), 4.24 (m, 1H, H-2'), 2.93, 2.71 (ABX, *J* = 14.1, *J'* = 7.8, *J''* = 4.7 Hz, 2H, H_2-1'), 2.10 (s, 3H, H-4'). **– ^{13}C NMR** (CD_3OD, 125 MHz) see Table 15. – **^{1}H,^{1}H COSY** and **HMBC** see Figure 140. **–** (+)-**ESIMS:** *m*/*z* = 203 $[M+Na]^+$, 383 $[2M+Na]^+$. – (-)-**ESIMS:** *m*/*z* = 179 $[M-H]^-$, 359 $[2M-H]^-$. – (+)-**HRESIMS:** *m*/*z* =

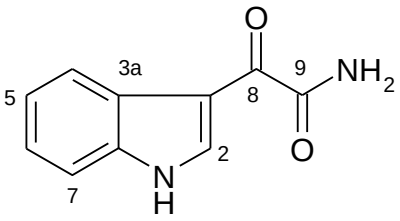
203.0681 $[M+Na]^+$ (calcd. 203.0679 for $C_{10}H_{12}NaO_3$). – (-)-**HRESIMS:** *m*/*z* = 179.0715 $[M-H]^-$ (calcd. 179.0714 for $C_{10}H_{11}O_3$).

N-Acetyl-2-aminophenol (92):



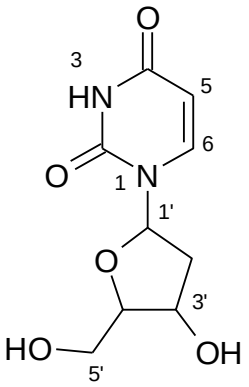
Colourless solid, 1.5 mg. , UV active at 254 nm, no colour with anisaldehyde/sulphuric. – $\boldsymbol{R_f}$ = 0.27 ($CHCl_3$/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.56 (dd, *J* = 7.9, *J* = 1.5 Hz, 1H, H-6), 6.98 (dt, *J* = 8.9, *J* = 1.6 Hz, 1H, H-4), 6.83 (dd, *J* = 8.1, *J* = 1.4 Hz, 1H, H-3), 6.77 (dt, *J* = 8.0, *J* = 1.5 Hz, 1H, H-5), 2.15 (s, 3H, $COCH_3$). – **EIMS** (70 eV)**:** *m*/*z* = 151 ($[M]^+$, 20), 109 ($[M- COCH_3]^+$, 100), 43 ($[COCH_3]^+$, 35).

Indolyl-3-glyoxylamide (**93**)



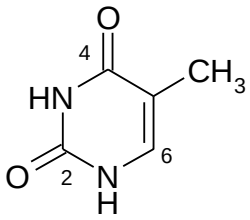
Colourless crystal, 1.5 mg, UV active at 254 nm, turned to red with anisaldehyde/sulphuric and heating. – $\boldsymbol{R_f}$ = 0.35 ($CHCl_3$/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.71 (s, 1H, H-2), 8.28 (d, *J* = 7.8 Hz, 1H, H-4), 7.46 (t, *J* = 7.6 Hz, 1H, H-5), 7.28- 7.20 (m, 2H, H-7, 6) – **^{13}C NMR** (CD_3OD, 125 MHz): δ 183.1 (CO-8), 168.2 (CO-9), 139.4 (CH-2), 137.9 (C_q-7a), 127.9 (C_q-3a), 124.8 (CH-6), 123.8 (CH-5), 122.9 (CH-4), 113.9 (C_q-3), 113.0 (CH-7). – (+)-**ESIMS:** *m*/*z* = 211 $[M+Na]^+$, 399 $[2M+Na]^+$. – (-)-**ESIMS:** *m*/*z* = 187 $[M-H]^-$. – **(+)-HRESIMS:** *m*/*z* = 211.0482 $[M+Na]^+$ (calcd for $C_{10}H_8N_2NaO_2$, 211.0478). – **(-)-HRESIMS:** *m*/*z* = 187.0513 $[M-H]^-$ (calcd for $C_{10}H_7N_2O_2$, 187.0513).

Deoxyuridine (94):



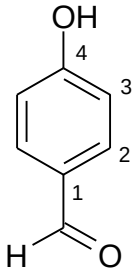
Colourless oily substance, UV absorbance at 254 nm, turned to blue colour with anisaldehyde/sulphuric and heating. − $\boldsymbol{R_f}$ = 0.15 (CH_2Cl_2/10% CH_3OH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.97 (d, J = 8.1, 1H, H-6), 6.25 (t, J = 6.8 Hz, 1H, H-1'), 5.68 (J = 8.1 Hz, 1H, H-5), 4.37 (m, 1H, H-4'), 3.91 (q, J = 6.9, J = 3.5, 1H, H-3'), 3.77 (dd, J = 12.0, J = 3.8 Hz , 1H, H5'-a), 3.70 (dd, J = 3.3, J = 12.0 Hz, 1H, H-5'b), 2.32 (m, 2H, H-2').

Thymine (95):



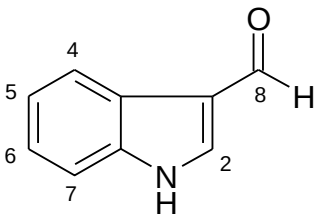
Colourless oily substance, UV absorbance at 254 nm turned to blue colour with anisaldehyde/sulphuric acid and heating. − $\mathbf{R_f}$ = 0.15 (CH_2Cl_2/10% CH_3OH). − **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 10.85 (sbr , 2H, NH-1, 3), 7.23 (s, 1H, H-6), 1.72 (s, 3H, 5-CH_3).

4-Hydroxybenzaldehyd (96):



Colourless solid substance, UV absorbance at 254 nm turned to yellow colour with anisaldehyde/sulphuric acid and heating, $\boldsymbol{R_f}$ = 0.70 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 9.75 (s, 1H, 1- CHO), 7.76 (d, J = 8.8 Hz, 2H, H-2, 2'), 6.90 (d, J = 8.6, 2H, H-3, 3').

Indole-3-carbaldehyde (97):



Colourless solid, turned to yellow with anisaldehyde/sulphuric acid reagent and heating, $\boldsymbol{R_f}$ = 0.75 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 9.86 (s, 1H, CHO), 8.14 (d, J = 7.2, 1H, CH-4), 8.08 (s,

1H, CH-2), 7.46 (dd, *J* = 6.9, *J* = 2.0 Hz, 1H, CH-7), 7.26 (t, *J* = 7.2 Hz, 1H, CH-5), 7.21 (t, *J* = 7.6 Hz, 1H, CH-6).

6.11 *Bacillus licheniformis*

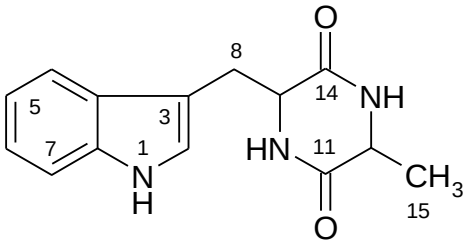
6.11.1 Fermentation and working up

A well-grown sub-culture of the *Bacillus licheniformis* was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed over XAD-16 and the latter afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. Due to a similar composition of both extracts, they were combined. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (7.8 g).

6.11.2 Scale up and isolation

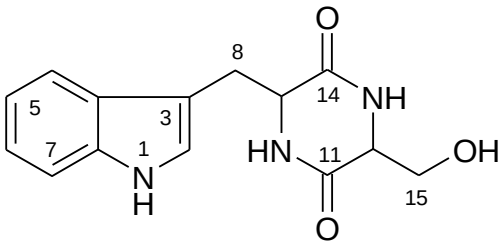
The strain was scale up to 25 l. The crude extract 3.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 2 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; four factions were selected for further investigation. Fraction II afforded Niax and tryptophol and 3-hydroxyacetyl indole, fraction III purified by Sephadex LH-20 eluted with methanol gave *cyclo*(Tyr,Pro) (**116**), *cyclo*(Pro,Val), *cyclo*(Ala,Pro) (**106**), *cyclo*(Phe,Gln) (**104**), Fraction IV was purified by using silica gel column to afford *cyclo*(Dehydroala,Ile) (**113**), cordycedipeptide A (**105**), Fraction V purified by Sephadex LH-20 eluted with methanol to afford *cyclo*(Ala,Trp) (**99**), *cyclo*(Ser,Trp) (**100**), Triethyl amine (**103**), Fraction VI was subjected to Sephadex LH-20 to afford *S*-methyl-adenosine (**102**); see Figure 145.

***Cyclo*(Ala,Trp) (99):**



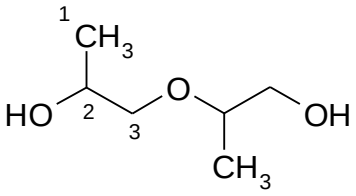
Colourless solid, UV active, turned to yellow with anisaldehyde/sulphuric acid, R_f = 0.75 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.58 (d, J = 8.0 Hz, 1H, H-4), 7.30 (d, J = 7.7 Hz, 1H, H-7), 7.05 (s, 1H, H-2), 7.08 (t, J = 7.8 Hz, 1H, H-6), 6,98 (t, J = 7.8 Hz, 1H, H-5), 4.26 (m, 1H, H- 9), 3.70 (m, 1H, H-12), 3.42 (m, 1H, H-8a), 3.17 (m, 1H, H-8b), 0.06 (d, 3H, CH_3-15). – **^{13}C NMR** (CD_3OD, 125 MHz): δ = 170.4 (C_q-14), 169.3 (C_q-11), 138.5 (C_q-7a), 129.1 (C_q-3a), 125.7 (CH-5), 122.4 (CH-2), 120.0 (CH-6), 119.9 (CH-4), 112.0 (C_q-3), 109.2 (CH-7), 57.5 (CH-9), 51.7 (CH-12), 30.8 (CH_2-8), 20.0 (CH_3-15).

***Cyclo*(Ser,Trp) (100):**



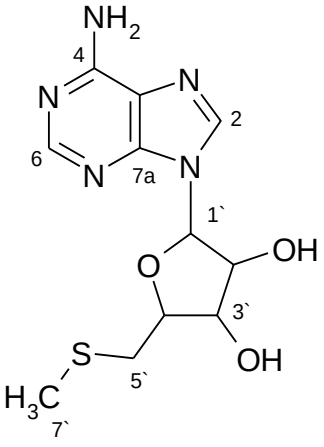
Colourless solid, UV active, turned to orange with anisaldehyde/sulphuric acid, R_f = 0.75 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.60 (dt, J = 7.9, J = 1.9 Hz, 1H, H-4), 7.33 (dt, J = 8.0, J = 1.6 Hz, 1H, H-7), 7.10 (s, 1H, H-2), 7.08 (tt, J = 8.0, J = 1.2 Hz, 1H, H-6), 7.00 (tt, J = 8.0, J = 1.2 Hz, 1H, H-5), 4.21 (dq, J = 4.1, J = 1.0 Hz, 1H, H-9), 3.81 (dq, J = 3.3, J = 1.1 Hz, 1H, H-12), 3.36 (m, 1H, H-15a), 3.30 (m, 2H, H_2-8), 2.88 (m, 1H, H-15b). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 170.0 (C_q-14), 167.9 (C_q-11), 137.9 (C_q-7a), 128.8 (C_q-3a), 126.0 (CH-5), 125.3 (CH-2), 122.4 (CH-6), 119.6 (CH-4), 112.2 (CH-7), 109.8 (C_q-3), 64.7 (CH_2-15), 58.8 (CH-12), 57.3 (CH- 9), 32.0 (CH_2-8). – **^{1}H,^{1}H COSY** and **HMBC** see Figure 154. – (+)-**ESIMS:** m/z = 296 $[M+Na]^+$, 569 $[2M+Na]^+$. – (-)-**ESIMS:** m/z = 272 $[M-H]^-$, 545 $[2M-H]^-$. – (+)-**HRESIMS:** m/z = 296.1009 $[M+Na]^+$ (calcd. 296.1006 for $C_{14}H_{15}N_3NaO_3$). – (-)-**HRESIMS:** m/z = 272.1035 $[M-H]^-$ (calcd. 272.1041 for $C_{14}H_{14}N_3O_3$).

Polypropylenglycol (**101**)**:**



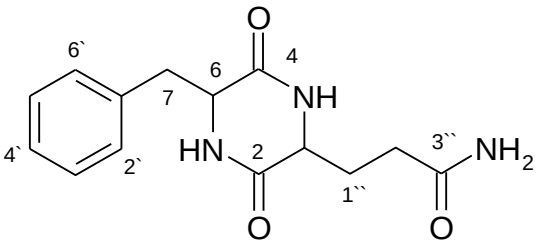
Colourless oil, 20.5 mg, UV inactive, turned to red with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.15 (5% CH_2Cl_2/MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 3.51 (m, 3H, [CH_2-3, CH-2]), 1.12 (d, J = 2.0 Hz, CH_3-1).

S'-Methyl-adenosine (102):



Colourless solid, UV active, turned to grey with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.43 ($CHCl_3$/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.31 (s, 1H, H-2), 8.19 (s, 1H, H-6), 5.99 (d, J = 5.1 Hz, 1H, H-1'), 4.77 (t, J = 5.2 Hz, 1H, H-2'), 4.31 (t, J = 4.7 Hz, 1H, H-3'), 4.21 (m, 1H, H-4'), 2.94 (ABX, J_{AB} = 14.2, J_{AX} = 5.6 Hz, 1H, 5'-H), 2.85 (ABX, J_{AB} = 14.2, J_{BX} = 6.1 Hz, 1H, 5'-H), 2.11 (s, 3H, 5'-SCH_3). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 153.8 (C_q-4), 157.2 (CH-6), 150.6 (C_q-7a), 141.2 (CH-2), 120.4 (C_q-7a), 90.0 (CH-1'), 85,5 (CH-5'), 74.9 (CH-3'), 74.0 (CH-2'), 37.5 (CH_2-6), 16.6 (CH_3-6).

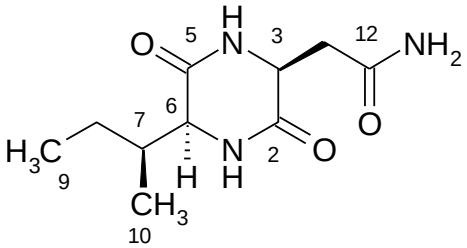
***Cyclo*(Phe,Gln) (104):**



Colourless solid, turned to orange with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.22 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (DMSO-d_6, 125 MHz): δ 8.07 (s br, 1H, NH-1), 8.00 (s br, 1H, NH-4), 7.27 (m, 2H, H-3', 5'), 7.18 (m, 3H, H-2', 4', 6'), 7.0, 6.6 (sbr, 2H, NH_2), 4.16 (t, 1H, J = 4.5 Hz, H-6), 3.64 (t, J = 5.7 Hz, 1H, H-3), 3.10, 2.89 (ABX, J_{AB} = 13.6, J_{AX} = 4.3, J_{BX} = 5.0 Hz, 2H, CH_2-7), 1.68 (t, J = 8.0 Hz, 1H, H-2''), 1.35 (m, 1H, H-1_a''), 1.02 (m, 1H, H-1_b''). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 173.2 (C_q-3''), 166.7 (C_q-2), 166.2 (C_q-5), 136.0 (C_q-1'), 130.0 (CH-2',6'), 127.8 (CH-3', 5'), 126.4 (CH-4'), 55.2

(CH-6), 53.3 (CH-3), 38.2 (CH_2-7), 30.2 (CH-2"), 29.3 (CH-1"). – **^{1}H,^{1}H COSY** see Figure 160, **HMBC** see Figure 162. – (+)-**ESIMS:** *m*/*z* = 573 $[2M+Na]^+$, 298 $[M+Na]^+$. – (-)-**ESIMS:** *m*/*z* = 274 $[M-H]^-$. – (+)-**HRESIMS:** *m*/*z* = 298.1164 $[M+Na]^+$ (calcd 298.1162 for $C_{14}H_{17}N_3NaO_3$).

Cordycedipeptide A (105):



Colourless solid, turned to yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.20 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 7.98 (s br, 1H NH-4), 7.68 (s, 1H, NH-1), 7.40 (s, 1H, NH_a-13), 6.91 (s, 1H, NH_b-13), 4.19 (m, 1H, CH-3), 3.77 (m, 1H, CH-6), 2.69 (m, 1H, CH_a-11), 231 (m, 1H, CH_b-11), 1.85 (m, 1H, H-7), 1.21 (m, 1H, H_a-8), 1.42 (m, 1H, H_b-8), 0.93 (d, *J* = 7.1 Hz, 3H, H-10), 0.85 (t, *J* = 7.3 Hz, 3H, H-9). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 171.8 (C_q-12), 167.4 (C_q-2)**,** 166.5 (C_q-5), 58.3 (CH-6), 50.8 (CH-6), 38.5 (CH_2-11), 37.5 (CH-7), 24.1 (CH_2-8), 15.0 (CH_3-10), 11.8 (CH_3-9). – (+)-**ESIMS:** *m*/*z* = 477 $[2M+Na]^+$, 250 $[M+Na]^+$. – (-)-**ESIMS:** *m*/*z* = 226 $[2M-H]^-$, 453 $[2M-H]^-$.

6.12 *Bacillus subtilis* MZ 6

6.12.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and moderate activity against Artemia *salina.*

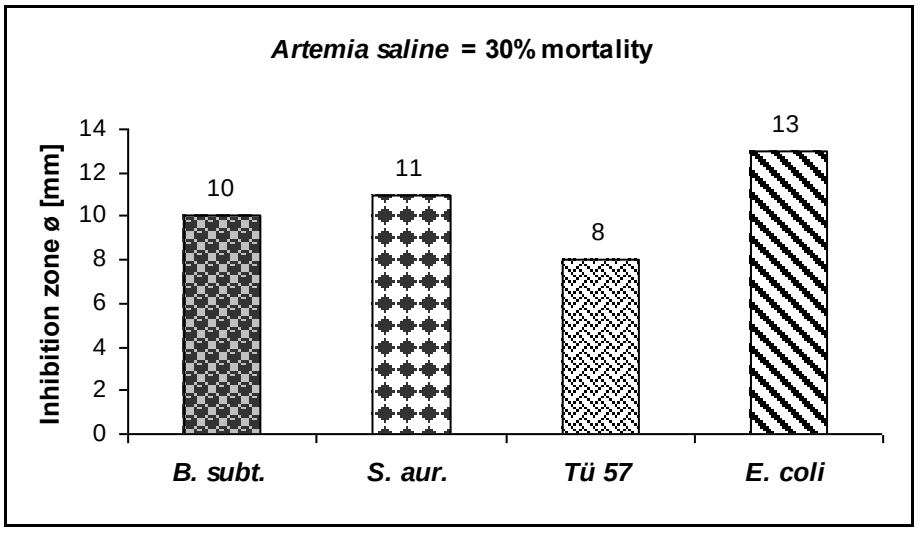


Figure 256: Biological activity of the crude extract from the *Bacillus subtilis* MZ 6 at 40 μg/paper disk

6.12.2 Fermentation and working up

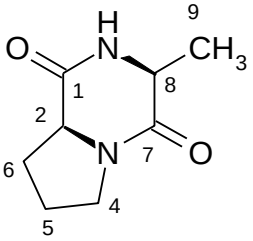
The strain *Bacillus subtilis* MZ 6 formed brown mycelial colonies. A 20-liter shaker culture of the *Bacillus* sp MZ 6 was incubating at 34 °C using LB medium at PH 7.0. The resulting brown culture broth was harvested after 5 days, mixed with ca. 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the aqueous filtrate and a mycelial fraction. The water phase was subjected to extraction separately using XAD-16 resin followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times) followed by acetone (3 times). The EtOAc and acetone phases were evaporated and dryness Scale up and isolation On TLC the three crude extracts showed similar zones, accordingly they were collected together, the extract was defatted with washing with cyclohexane to get 4.5 g of brownish crude extract.

6.12.3 Scale up and isolation

The crude extract (4.5 g) subjected to silica gel column chromatography using CH_2Cl_2/MeOH gradient (column 3 x 60 cm, 0 to 20 % MeOH), three fractions were selected for further investigation. Fraction II was purified on Sephadex LH-20 using MeOH to afford cis *cyclo*(Ala,Pro) (**106**) and N-(4-oxo-pentyl)-acetamide (**107**) , F III delivered acetyl tryptamine (**108**), tryptophane and tyrosol (**109**)**.** Fraction IV sub-

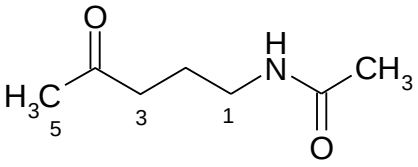
jected to Sephadex LH-20 followed by RP-18 using MeOH/H_2O to deliver KF8940 (**110**) , see Figure 164.

Cyclo(**Ala,Pro**) (**106**):



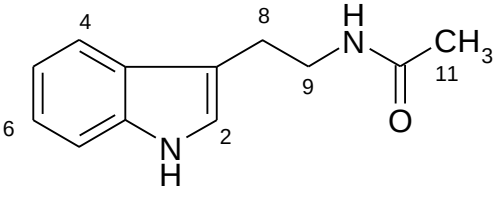
Colourless solid, UV inactive, turned to yellow with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.22 (m, 2H, H-2, 8), 3.50 (m, 2H, H_2-4), 2.28 (m, 1H, H-6a), 1.99 (m, 3H, H-6b, H_2-5), 1.37 (d, 3H, H_3-9). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 172.6 (CO-1), 169.0 (CO-7), 60.5 (CH-2), 52.1 (CH-8), 46.4 (CH_2-4), 29.2 (CH_2-6), 23.6 (CH_2-5), 15.7 (CH_3-9).

N-(4-Oxo-pentyl)-acetamide (107):



Colourless solid, UV inactive, turned to yellow with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.15 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 7.74 (sbr, 1H, NH), 2.98 (q, J = 6.9, 2H, H_2-1), 2.41 (t, J = 7.3 Hz, 2H, H_2-3), 2.06 (s, 3H, CH_3-5), 1.77 (s, 3H, $NHCOCH_3$), 1.57 (m, 2H, H_2-2). – **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 207.9 (CO-4), 169.0 (NHCO), 40.0 (CH_2-3), 37.8 (CH_2-1), 29.6 (CH_3-5), 22.9 (CH_2-2), 21.7 (NAc). – (+)-**ESIMS:** m/z = 144 $[M+H]^+$, 166 $[M+Na]^+$, 309 $[2M+Na]^+$. – (+)-**HRESIMS:** m/z = 144.1026 $[M+H]^+$ (calcd. 144.1019 for $C_7H_{13}NO_2$), 166.0847 $[M+Na]^+$ (calcd 166.0838 for $C_7H_{13}NNaO_2$). – (-)-**HRESIMS:** m/z = 142.0874 $[M-H]^-$(calcd 142.0874 for $C_7H_{12}NO_2$).

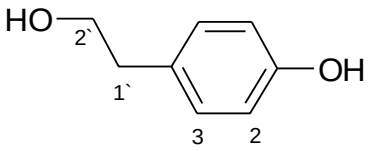
N_b-Acetyl tryptamine (108):



Colourless solid, UV absorbance, turned to blue with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.50. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.53 (d, J = 7.8 Hz, 1H, H-4), 7.32 (d, J =

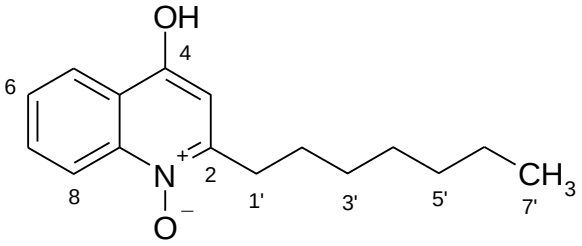
8.0 Hz, 1H, H-7), 7.07 (d, *J* = 7.0 Hz, 1H, CH-6), 7.00-6.95 (m, 2H, H-5, 2), 3.41 (dt, *J* = 11.4, *J* = 3.9 Hz 2H, H_2-9), 2.89 (t, *J* = 7.1 Hz, 2H, H_2-8), 1.85 (s, 3H, H_3-11).

Tyrosol (109):



Colourless oil, UV active, turned to red with anisaldehyde/sulphuric acid and heating. − $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.01 (d, *J* = 8.4 Hz, 2H, H-3, 5), 6.69 (d, *J* = 8.5 Hz, 2H, H-2, 6), 3.67 (t, *J* = 7.0 Hz, 1H, H-2'), 2.70 (t, *J* = 7.0 Hz, 1H, H-1')

2-Heptyl-4 (1H)-quinolinone-N-oxide (110):



Colourless solid, UV active, turned to yellow with anisaldehyde/sulphuric acid. − $\boldsymbol{R_f}$ = 0.45 (CH_2Cl_2/10% MeOH). − **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.25 (d, *J* = 8.0 Hz ,1H, H-5), 8.08 (d, *J* = 8.0 Hz , 1H, H-8), 7.58 (t, *J* = 8.4 , 1H, H-6), 7.36 (t, *J* = 8.0, 1H, H-7), 6.37 (s, 1H, H-3), 2.77 (m, 2H, H_2-1'), 1.77 (m, 2H, H_2-2'), 1.45- 1.18 (m, 8H, H_2-3', 4', 5' and 6'), 0. 86 (t, 3H, H_3-7'). – **^{13}C NMR** (125 MHz, CD_3OD), 174.0 (CO-4), 156.4 (C_q-2), 142.0 (C_q-8a), 133.6 (CH-7), 126.0 (CH-6), 125.9 (CH-5), 125.4 (C_q-4a), 116.8 (CH-8), 107.5 (CH-3), 32.9 (CH_2-4'), 32.6 (CH_2-1'), 30.4 (CH_2-3'), 30.1 (CH_2-5'), 28.9 (CH_2-2'), 23.7 (CH_2-6'), 14.4 (CH_3-7'). – **^{1}H,^{1}H COSY** see Figure 177, **HMBC** see Figure 179. – (+)-**ESIMS:** *m*/*z* = 260 $[M+H]^+$, 282 $[M+Na]^+$, 519 $[2M+H]^+$. − (-)-**ESIMS:** *m*/*z* = 258 $[M-H]^-$, 517 $[2M-H]^-$. – (+)-**HRESIMS:** *m*/*z* = 260.1648 $[M+H]^+$ (calcd. 260.1645 for $C_{16}H_{21}NO_2$).

6.13 Terrestrial *Streptomyces* sp. N859

6.13.1 Pre-screening

The terrestrial *Streptomyces* sp. N859 was isolated and identified by Prof. Wolf. TLC showed several UV active zones, which turned to orange, pale yellow and violet with anisaldehyde/sulphuric. Due to the interesting colour reaction and UV activity the strain was selected.

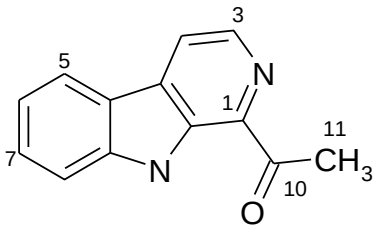
6.13.2 Fermentation and working up

A well-grown sub-culture of the Terrestrial *Streptomyces* sp. N859 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (4.2 g).

6.13.3 Scale up and Isolation

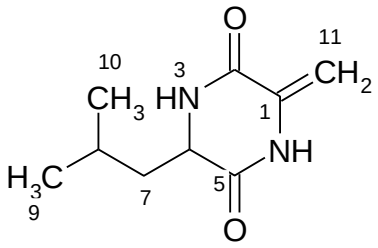
The crude extract was subjected to silica flash column chromatography, eluting with a step gradient solvent system of Dichlormethan and methanol, to afford three fractions. Fraction II was further separated by passage over silica flash column eluted with cyclohexane - ethyl acetate (10 to 100%) to give three subfractions (FIIa-FIIc). Fraction IIa and IIc were separated using PTLC with CH_2Cl_2: CH_3OH (95: 5) to afford *cyclo*(Dehydroala,Leu) (**113**) and *cis-cyclo*(Tyr,Pro) (**116**)**.** Fraction IIb was further purified by Sephadex LH-20 eluted with methanol to give indole-3-carboxylic acid as well as to another to sub fractions (FIIb1 and FIIb2). Fraction IIb1 was washed with CH_2Cl_2 to give *cyclo*(Ala-Ile) (**114**) while fraction IIb2 was purified over PTLC with CH_2Cl_2: CH_3OH (95: 5) to afford compound *trans-cyclo*(Tyr-Pro) (**115**). Purification of fraction III afforded anthranilic acid, 3-hydroxyacetylindole (**117**) and 1-acetyl-*β*-carboline (**111**); see Figure 181**.**

1-Acetyl-*β*-carboline (111):



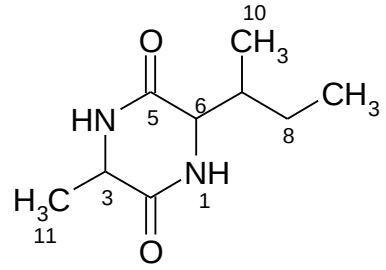
It showed on TLC pale yellow with anisaldehyde/sulphuric acid and Ehrlich's reagent. – $\boldsymbol{R_f}$ = 0.90 ($CHCl_3$/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 10.31 (s br, 1H), 8.54 (d, J = 5.1 Hz, 1H, H-3), 8.16 (d, J = 5.1 Hz, 1H, H-4), 8.15 (m, J = 5.1 Hz, 1H, H-5) 7.62 (m, 2H, H-7, 8), 7.31 (m, 1H, H-6), 2.91 (s, 3H, CH_3-11). – (+)-**ESIMS:** m/z = 211 $[M+H]^+$. – **(-)-ESIMS:** m/z = 209.3 $[M-H]^-$.

***Cyclo*(Dehydroala,Leu) (113):**



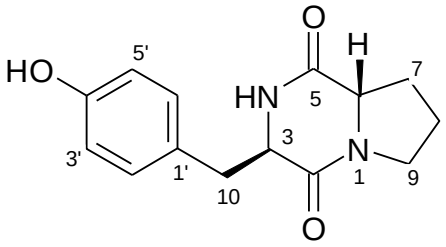
Colourless solid, UV-absorbing at 254 nm, coloured to orange by anisaldehyde/sulphuric acid reagent and Ehrlich reagent. – $\boldsymbol{R_f}$ = 0.51 (CH_2Cl_2/10% MeOH).– **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 10.47 (s br, 1H, NH-1), 8.40 (s br, 1H, NH-4), 5.18 (s, 1H, Ha-11), 4.79 (s, 1H, Hb-11), 3.96 (t, ^3J = 8.0 Hz, 1H, H-6), 1.80 (m, 1H, H-8), 1.58 (m, 2H, H-7), 0.86 (d, J = 6.5 Hz, 6H, H-9,10). – **^{13}C NMR** (DMSO-d_6, 500 MHz): δ 166.3 (CO-5), 158.0 (CO-2), 134.5 (C_q-3), 98.8 (CH_2-11), 53.7 (CH-6), 43.5 (CH_2 -7) 23.4 (CH-8), 22.6 (CH_3-9), 22.1 (CH_3-10). – (-)-**ESIMS:** m/z = 385.0 $[2M-2H+Na]^-$, 181.1 $[M-H]^-$.

***Cyclo*(Ala,Ile) (114):**



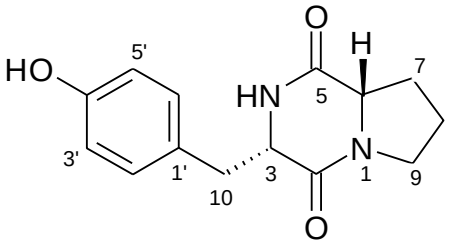
Colourless solid, coloured to violet by anisaldehyde/sulphuric acid and heating, blue with chlorine/anisidine. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.01 (m, 1H, H-6), 3.91 (m, 1H, H-3), 1.86 (m, 1H, H-7), 1.67 (m, 2H, H-8), 1.44 (d, J = 7.1 Hz, 3H, H-11), 0.97 (d, J = 6.4 Hz, 3H, H-10), 0.96 (t, J = 5.5 Hz, 3H, H-9). – **(+)-ESIMS:** m/z = 185 $[M+H]^+$. – (+)-**ESIHRMS:** m/z = 185.12904 ($[M+H]^+$, 185.12900 calcd. for $C_9H_{17}N_2O_2$).

***Trans-Cyclo*(Tyr,Pro) (115)**



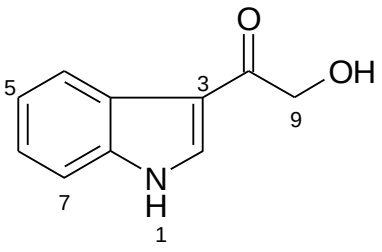
Colourless solid (10 mg), UV absorbing zone substance, stained to violet with anisaldehyde/sulphuric acid and pink with Ehrlich's reagent, as well as blue with chlorine/anisidine reaction. – $\boldsymbol{R_f}$ = 0.82 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.97 (d, J = 8.6 Hz, 2H, H-2', 6'), 6.71 (d, J = 8.6 Hz, 2H, H-3', 5'), 4.14 (t, J = 5.3 Hz, 1H, H-3), 3.51 (m,1H, H-6), 3.30 (m, 1H, Ha-9), 3.09 (dd, J = 13.8, J = 4.4 Hz, 1H, Ha-10), 2.87 (dd, J = 13.8, J = 4.6 Hz, 1H, Hb-10), 2.62 (m, 1H, Hb-9), 2.04, 1.87, 1.63 (3m, 4H, H-7,8). – (+)-**ESIMS:** m/z = 261.1 $[M+H]^+$. – (-)-**ESIMS:** m/z = 519.0 $[2M-H]^-$, 259.2 $[M-H]^-$.

***Cis-Cyclo*(Tyr-pro) (116)**



Colourless solid, violet with anisaldehyde/sulphuric acid and heating and pink with Ehrlich's reagent. − $\boldsymbol{R_f}$ = 0.73 (CH_2Cl_2/7% CH_3OH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.03 (d, J = 8.7 Hz, 2H, H-2', 6'), 6.69 (d, J = 8.6 Hz, 2H, H-3', 5'), 4.35 (td, J = 4.7, J = 1.9 Hz, 1H, H-3), 4.03 (m, 1H, 6-H), 3.60-3.33 (m, 2H, CH_2-9), 3.04 (m, 2H, H-10), 2.10, 1.79, 1.22 (3m, 4H, CH_2-7, 8). **–** (+)-**ESIMS:** m/z = 565 $[2M-H+2Na]^+$, 542 ($[2M+Na]^+$, 283 ($[M+Na]^+$, 50). – (-)-**ESIMS:** m/z = 510 $[2M-H]^-$, 259 $[M-H]^-$.

3-Hydroxyacetylindole (117):



Orange solid, UV absorbing, brown and pale red with anisaldehyde and Ehrlich reagent respectively. – $\boldsymbol{R_f}$ = 0.28 (CH_2Cl_2/MeOH 95: 5). – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 8.94 (sbr, 1H, NH-1) 8.28 (m, 1H, H-4), 7.93 (d, ^3J = 6.5, 1H, H-2), 7.47 (m,

1H, H-7), 7.34 (m, 2H, H-5, 6), 4.79 (s, 2H, H-9). – **EIMS** (70 eV): *m/z* (%) = 175 ([M]•+, 24), 144 (100), 116 (18), 89 (12).

6.14 Marine derived *Streptomyces* sp. B7547

6.14.1 Pre-screening

The crude extract showed in the agar diffusion test activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and moderate activity against *Artemia salina.*

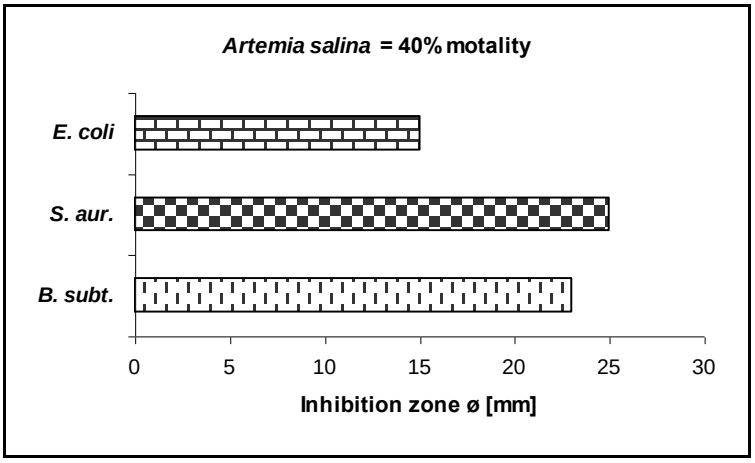


Figure 257: The biological activity for Marin derived *Streptomyces* sp. B7547 at 40 μg/paper disk

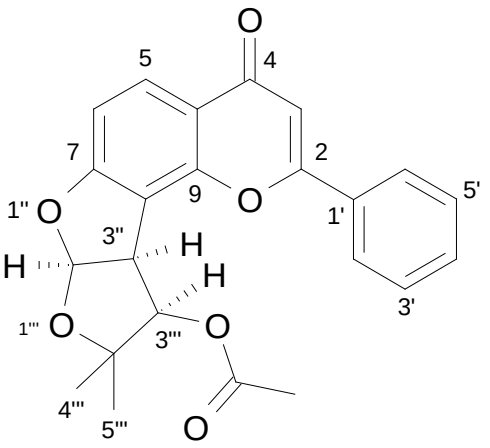
6.14.2 Fermentation and work-up

The strain Marin derived *Streptomyces* sp. B7547 formed yellow mycelial colonies. A 25-liter shaker culture of the marine derived *Streptomyces* sp. B7547 was incubated a 28 °C using M_2^+ medium with 50% seawater. The fermentor broth was harvested after 7 days, mixed with Celite, and then filtered. The filtrate and mycelia were subjected to extraction separately using XAD-16 for the water phase, followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times).

6.14.3 Scale up and isolation

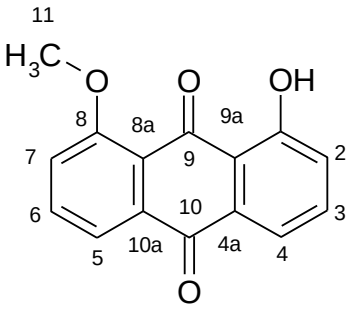
The strain was scale up in to 25 l. The crude extract 3.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 2 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; two factions were selected for further investigation. F II afforded pseudosemiglabrin (**118**) from silica gel column chromatography using CH_2Cl_2/MeOH followed by Sephadex LH-20 using MeOH and a mixture of semiglabrin and PHB, F III afforded 1-hydroxy-8-methoxy-anthraquinone (**119**) after purification by Sephadex LH-20 using MeOH; see Figure 189.

Pseudosemiglabrin (118):



Colourless solid, UV absorbing at 254 nm turned to blue-green colouration with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.40 (CH_2Cl_2/5% MeOH)**.** – **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.04 (d, J = 8.7 Hz, 1H, H-5), 7.93 (dd, J = 7.8, J = 1.3 Hz, 2H, H-2', 6'), 7.58- 7.49 (m, 3H, H-3', 5', H-4'), 6.94 (d, J = 8.6 Hz, 1H, H-6), 6.80 (s, 1H, H-3), 6.50 (d, J = 6.5 Hz, 1H, H-2"), 5.60 (d, J = 8.8 Hz, 1H, H-3'"), 4.74 (dd, J = 8.8, J = 6.5 Hz, 1H, H-3"), 1.42 (s, 3H, OAc-3'"), 1.35 (s, 3H, H-4'"), 1.08 (s, 3H, H-5'"). – **^{13}C NMR** (CD_3OD, 300 MHz): δ 179.6 (C_q-4), 170.9 (COO-3'"), 166.3 (C_q-7), 164.8 (C_q-2), 155.2 (C_q-9), 133.0 (CH, C-4'), 132.3 (C_q-1'), 130.1 (CH-3', 5'), 129.1 (CH-5), 127.5 (CH-2', 6'), 118.8 (C_q-10), 113.7 (CH-2"), 113.5 (C_q-8), 110.1 (CH-6), 107.5 (CH-3), 85.9 (C_q-2'"), 78.2 (CH-3'"), 49.0 (CH-3"), 27.8 (CH_3-4'"), 23.6 (CH_3-5'"), 20.4 ($COCH_3$-3'").

1-Hydroxy-8-methoxy-anthraquinone (119):



Yellow crystals, UV active, yellow with praying with anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz): δ 12.93 (sbr, 1H, OH), 7.95 (dd, J = 7.7, J = 1.0 Hz, 1H, H-5), 7.75 (dd, J = 7.5, J = 1.2 Hz, 1H, H-2), 7.73 (tt, J = 7.5, J = 1.2 Hz, 1H, H-6), 7.60 (t, J = 7.8, J = 1.6 Hz, 1H, H-3), 7.35 (d, J = 8.4 Hz, 1H, H-7), 7.27 (dd, J = 8.3, J = 1.2 Hz, 1H, H-4), 4.1 (s, 3H, OCH_3). – **^{13}C NMR** ($CDCl_3$, 300 MHz): δ 188.7 (C_q-9), 182.7 (C_q-10), 162.5 (C_q-1), 160.7 (C_q-8), 135.8 (CH-6), 135.7 (C_q-5a), 135.7 (CH-3), 132.6 (C_q-4a), 124.7 (CH-4), 120.7 (CH-8a), 118.8 (CH-2), 118.1 (CH-7),117.0 (C_q-9a), 56.7 (CH_3-11).

Mixture of two glycosides (120):

Colourless oily substance turned to brown colour with anisaldehyde sulphuric acid, $\boldsymbol{R_f}$ = 0.25 **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.61 (m, 1H, H-5), 5.31 (dd, J = 16.5, J = 3.6, 1H, H-1'), 4.7 (ddd, J = 2.2, J = 2.1, J = 2.2), 4.28 (m, 1H), 3.82-3.29 (m, 9H). – (+)-**ESIMS** *m/z* 329 $[M+Na]^+$, 635 $[2M+Na]^+$. – (-)-**ESIMS:** *m/z* = 305 $[M-H]^-$, 611$[2M-H]^-$. – (+)-**HRESIMS:** *m/z* = 329.08433 (calcd. 329.08430 for $C_{12}H_{18}NaO_9$).

6.15 Terrestrial *Streptomyces* sp. GW 7/186

6.15.1 Pre-screening

The crude extract of the terrestrial *Streptomyces* sp. GW 7/186 showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Mucor miehei* and *Artemia salina.*

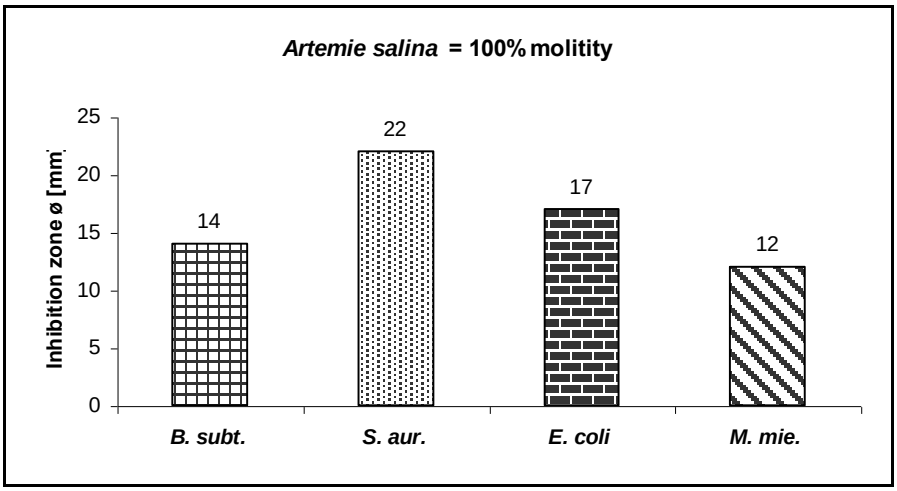


Figure 258: Biological activity for the crude extract of the terrestrial *Streptomyces* sp. GW 7/186

6.15.2 Fermentation and working up

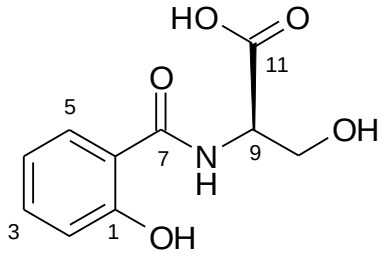
A well-grown sub-culture of the of the terrestrial *Streptomyces* sp. GW 7/186 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a brown culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (3.5 g).

6.15.3 Scale up and isolation

The crude extract 3.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 2 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; two factions were selected for further investigation. Fraction II purified by Sephadex LH-20 eluted with methanol to afford madurastatin B2 (**121**) and tetralone **122** while Sephadex LH-20 purified

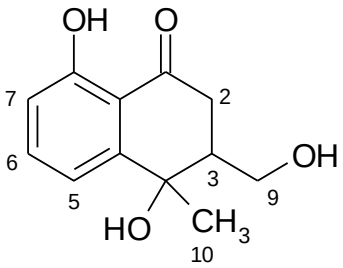
Fraction III followed by RP-18 using MeOH/H_2O to deliver two primary metabolism adenosine and uracil, see Figure 202.

Madurastatin B2 (121):



Colourless solid, UV absorbance, turned to green dark with anisaldehyde/sulphuric acid, $\boldsymbol{R_f}$ = 0.18 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (300 MHz, DMSO-d_6): δ 7.80 (dd, J = 7.9, J = 1.6 Hz, 1H, H-5), 7.31 (t, J = 8.5, J = 1.6 Hz, 1H, H-3), 6.90 (d, J = 8.3 Hz, 1H, H-2), 6.82 (t, J = 7.8, J = 0.8 Hz, 1H, H-4), 4.06 (t, J = 13.2, J = 6.4 Hz, 1H, H-9), 3.70 (ABX, J_{AB} = 9.8, J_{AX} = 5.6 Hz, 1H, H-10), 3.54 (ABX, J_{AB} = 9.8, J_{BX} = 6.8 Hz, 1H, H-10). – **^{13}C NMR** (125 MHz, DMSO-d_6): δ 172.9 (CO-11), 166.5 (CO-7), 158.9 (C_q-1), 132.6 (CH-3), 117.1 (C_q-6), 128.5 (CH-5), 117.9 (CH-4), 117.1 (CH-2), 55.5 (CH-9), 62.5 (CH_2-10). **^{1}H,^{1}H COSY** see Figure 205, **HMBC** see Figure 207. – (-)-**ESIMS**: *m/z* = 224.0 $[M-H]^-$. − (+)-**HRESIMS**: *m/z* = 248.05303 (calcd. 248.05294 for $C_{10}H_{11}N_1NaO_5$).

Tetralone 122:



Colourless oil, UV absorbance substance, turned to yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.50 (t, J = 8.3 Hz, 1H, H-6), 7.16 (d, ^3J = 7.7, 1H, H-7), 6.80 (d, ^4J = 8.4 Hz, 1H, H-5), 3.87 (ABX, J_{AB} = 11.1, J_{AX} = 5.6 Hz, 1H, H-9), 3.43 (ABX, J_{AB} = 11.1, J_{BX} = 8.0 Hz, 1H, H-9), 2.91 (m, 1H, H-3), 2.36 (m, 2H, H_2-2), 1.60 (s, 3H, H_3-10). – (+)-**ESIMS**: *m/z* = 245 $[M+Na]^+$. – (-)-**ESIMS**: *m/z* = 421 $[M-H]^-$

6.16 Terrestrial *Streptomyces* sp. MH4

The crude extract showed in the agar diffusion test activity against *Escherichia coli*, *Staphylococcus aureus*

Table 25: Antimicrobial activity of the crude extract of terrestrial *Streptomyces sp.* MH4

Extract	Agar diffusion test (40 μg/disc (∅ 5 mm); Diameter of inhibition zones in [mm]		
	E. coli	*Sac. cerv.*	*S. aur.*
MH4	13	10	12

6.16.1 Fermentation and working up

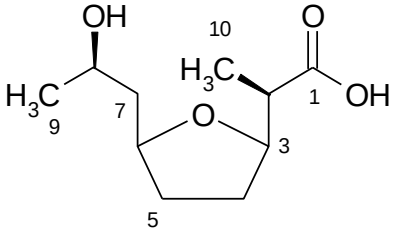
A well-grown sub-culture of the terrestrial *Streptomyces* sp. MH4 was used for inoculation of a 25 l shaker culture on M_2 medium; the pH was adjusted to 7.80 before sterilisation. After 7 days cultivation at 28 °C, the stain showed a black culture broth. The fermentor broth was filtered with a filter press. The filtrate was passed through over XAD-16 and afterwards extracted with methanol. The methanol phase was evaporated in *vacuo* and the remaining water was extracted three times with ethyl acetate. The biomass phase was extracted with ethyl acetate. The combined extracts were filtered and concentrated under vacuum to obtain a crude extract (6.1 g).

6.16.2 Scale up and isolation

The crude extract (6.1 g) was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH). Three fractions were selected for further investigation. Fraction II subjected to Sephadex LH-20 delivered nonactic acid (**123**) and homononactic acid (**124**). Fraction III was purified on Sephadex LH-20 using MeOH to afford 3-(3,3-di-indole)propane-1,2-diol (**125**) and turbomycin A (**126**). Fraction FIV was purified on Sephadex LH-20 using MeOH followed by RP-

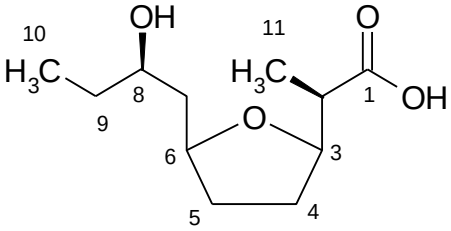
18 using MeOH/H_2O gradient (10 to 30 % MeOH) to deliver trivial compounds and primary metabolites; see Figure 209.

Nonactic acid (**123**):



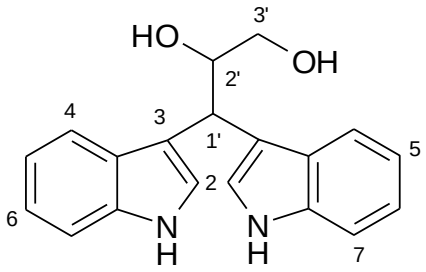
Colourless oil, UV inactive stained to violet by anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.36 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 3.99 (m, 2H, H-8, 6), 3.89 (m, 1H, H-3), 2.42 (dt, J = 13.4, J = 6.8 Hz, 1H, H-2), 1.99 (m, 2H, CH_2-7), 1.58 (m, 4H, CH_2-4, 5), 1.15 (d, J = 6.2 Hz, 3H, CH_3-9), 1.09 (d, J = 6.9 Hz, 3H, CH_3-10). – **^{13}C NMR** (CD_3OD, 125 MHz): δ 179.2 (C_q-1), 82.0 (CH-3), 77.9 (CH-6), 66.1 (CH-8), 46.1 (CH-2), 32.2 (CH-5), 29.4 (CH-4), 24.1 (CH_3-9), 14.1 (CH_3-10). – (+)-**ESIMS**: *m/z* = 225 $[M+Na]^+$, 427 $[2M+Na]^+$. – (-)-**ESIMS**: *m/z* = 201 $[M-H]^-$, 403 $[2M-H]^-$.

Homononactic acid (124):



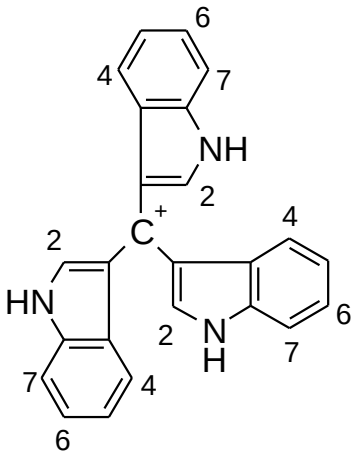
UV inactive, colourless oil, turns to violet with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.39 (CH_2Cl_2/5% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 4.02 (m, 2H, H-8, 6), 3.64 (m, 1H, H-3), 2.42 (m, 1H, H-2), 1.99-120 (m, 8H, H-4, 5, 7, 9), 1.09 (d, J = 6.9, 3H, CH_3-11), 0.91 (t, 3H, CH_3-10). – **^{13}C NMR** (CD_3OD, 125 MHz), 179.2 (C_q-1), 81.8 (CH-3), 77.8 (CH-6), 71.1 (CH-8), 46.9 (CH-2), 43.8 (CH_2-7), 32.1 (CH_2-5), 31.4 (CH_2-9), 29.3 (CH_2-4), 13.9 (CH_3-11), 10.17 (CH_3-10).

3-(3,3-Di-indole)propane-1,2-diol (125):



red oil, UV absorbance, turned to red with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.19 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 7.54 (dd, J = 7.9, J = 2.7 Hz, 2H, H-4), 7.28 (dd, J = 8.1, J = 1.0 Hz, 2H, H-7), 7.29 (s, 2H, H-2), 7.01 (dt, J = 7.0, J = 4.7 Hz, 2H, H-6), 6.90 (dt, J = 8.9, J = 1.0 Hz, 2H, H-5), 4.68 (d, J = 6.7 Hz, 1H, H-1'), 4.48 (dt, J = 7.0, J = 4.1 Hz, 1H, H-2'), 3.61 (ABX, J_{AB} = 11.1, J_{AX} = 4.1 Hz, 1H, H-3'). 3.48 (ABX, J_{AB} = 11.1, $^3J_{BX}$ = 7.1 Hz, 1H, H-3'). – (+)-**ESIMS**: *m/z* = 329 $[M+Na]^+$, 635 $[2M+Na]^+$. – (-)-**ESIMS**: *m/z* = 305 $[M-H]^-$. – (+)-**HRESIMS**: *m/z* = 329.1263 (calcd. 329.1260 for $[M+Na]^+$).

Turbomycin A (126):



Red oily substance, UV active turned to red by spraying with anisaldehyde regent and heating. – $\boldsymbol{R_f}$ = 0.27 (CH_2Cl_2/ 7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 8.24 (s, 3H, H-2), 7.64 (d, J = 9.5 Hz, 3H, H-4), 7.28 (t, J = 7.6 Hz, 3H, H-5), 7.01 (t, J = 7.5 Hz, 3H, H-6), 6.90 (d, J = 8.4 Hz, 3H, H-7). – (+)-**ESIMS**: *m/z* = 360.2 $[M+H]^+$. – (-)-**ESIMS**: *m/z* = 358.1 $[M-H]^-$. − (+)-**HRESIMS**: *m/z* = 360.1501 (calcd. 360.1495 for $[M+H]^+$, $C_{25}H_{18}N_3$).

6.17 *Trichoderma* sp.

6.17.1 Pre-screening

The crude extract showed in the agar diffusion test antimicrobial activity listed in the next table.

Table 26: The biological activity for *Trichoderma* sp.

Fungal Extract	Agar diffusion test (40 μg/disc (∅ 5 mm); Diameter of inhibition zones in [mm]				
	E. coli	*PS*	*B. subt.*	*S. aur.*	*Asp. niger*
Supernatant	8	8	7	8	9
Mycelia	13	10	11	12	8

6.17.2 Fermentation and work-up

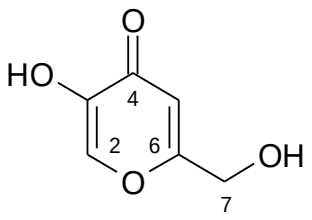
The strain *Trichoderma* sp formed green mycelial colonies. A 25-liter shaker culture of the *Trichoderma* sp was to incubate a 28 °C using M_2^+ with agar medium. The fermentor broth was harvested after 7 days, mixed with Celite, and then filtered. The filtrate and mycelia were subjected to extraction separately using XAD-16 for the water phase, followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times).

6.17.3 Scale up and isolation

The strain was scale up in to 25 l. The crude extract 7.5 g was dissolved in a mixture of CH_2Cl_2/MeOH and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3 x 75 cm, 150 g) chromatography (CH_2Cl_2/MeOH gradient, 1.5 l CH_2Cl_2, 1 l CH_2Cl_2/1% CH_3OH, 1 l CH_2Cl_2/2% CH_3OH, 2 l CH_2Cl_2/3% CH_3OH, 2 l CH_2Cl_2/5% CH_3OH, 1 l CH_2Cl_2/10% CH_3OH, 500 ml CH_2Cl_2/20% CH_3OH), under TLC control; two factions were selected for further investigation. F II afforded ergosterol (**128**) and ergosterol peroxide (**129**) from Sephadex column LH-20 eluted by MeOH, F III delivered kojic acid (**127**) and α-cyclopiazonic acid (**130**); see Figure 215.

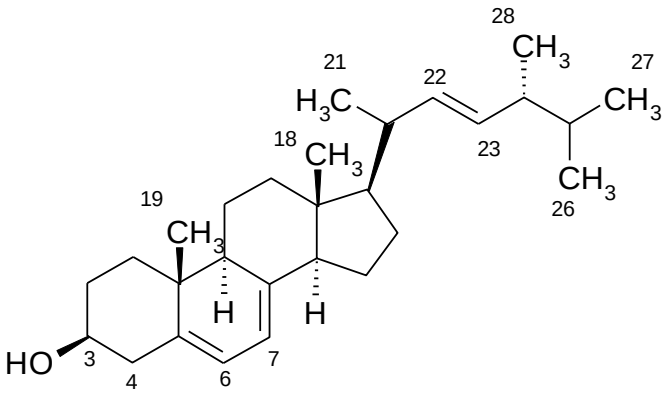
Kojic acid (127) :

UV absorbing at 254 nm, colourless solid, turned to blue colour by anisaldehyde/sulphuric after heating. $\boldsymbol{R_f}$ = 0.75 ($CHCl_3$/5% MeOH): – **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 8.99 (br, 1H, OH), 5.69 (br, 1H, OH), 7.97 (d, J = 8.0 Hz,



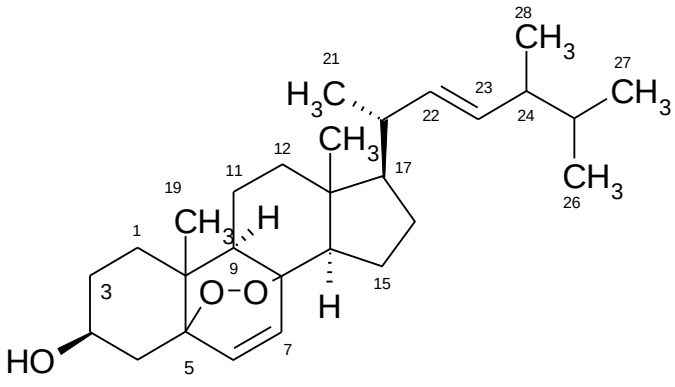
1H, CH-2), 6.34 (s, 1H, CH-5), 4.28 (s, 2H, CH_2-7). – **^{13}C/APT NMR** (DMSO-d_6, 125 MHz): δ 174.2 (C_q-4), 168.2 (C_q-6), 153.0 (C_q-3), 138.9 (CH-2), 110.0 (CH-5), 59.7 (CH_2-7).

Ergosterol (128):



Colourless solid, UV absorbance, turned to pink with spraying with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.28 (CH_2Cl_2/7% MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz): δ 5.24 (d, J = 8.5 Hz, 1H, H-22), 5.26 (d, J = 8.5 Hz, 1H, H-23), 5.48 (dd, J = 5.6, J = 2.4 Hz, 1H, H-6), 5.34 (dd, J = 5.4, J = 2.7 Hz, H-7), 3.40 (m, 1H, H-3), 2.35 (m, 1H, H-4a), 2.17 (t, 1H, H-4b), 2.60 (m, 1H, H-15), 2.00-1.17 many protons overlapped, 0.95 (d, J = 6.7 Hz, 3H, H_3-21), 0.94 (s, 3H, H_3-19), 0.91 (d, J = 7.0 Hz, 3H, H_3-28), 0.85 (d, J = 3.7 Hz, 3H, H_3-26), 0.82 (d, J = 3.7 Hz, 3H, H_3-27),0.62 (s, 3H, H_3-18). – (+)-**ESIMS**: m/z = 419 $[M+Na]^+$, 815 $[2M+Na]^+$

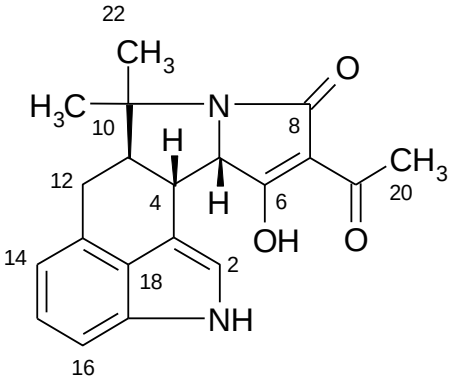
Ergosterol peroxide (129):



Colourless solid, turned to violet by spraying with anisaldehyde and heating. – $\boldsymbol{R_f}$ = 0.12. – **^{1}H NMR** (CD_3OD, 300 MHz): δ 6.52 (d, J = 8.5 Hz, 1H, H-6), 6.24 (d, J = 8.5 Hz, 1H, H-7), 5.20 (2H, J = 15.5, J = 7.5 Hz, 1H, H-22, 23), 3.76 (m, 1H, H-3), 2.21 (t, J = 7.5 Hz, 2H, H-4), 0.95 (d, J = 6.7 Hz, 3H, H_3-21), 0.94 (s, 3H, H_3-19), 0.91 (d, J = 6.9 Hz, 3H, H_3-28), 0.85 (d, J = 3.7 Hz,

3H, H_3-26), 0.79 (d, *J* = 3.7 Hz, 3H, H_3-27), 0.75 (s, 3H, H_3-18). – **(+)-ESIMS**: *m/z* = 451 $[M+Na]^+$, 879 $[2M+Na]^+$, 1308 $[3M+Na]^+$.

***α*-Cyclopiazonic acid (130)**:



Colourless crystal**,** UV absorbing substance, turned to red with anisaldehyde/sulphuric acid and heating. – **^{1}H NMR** (CD_3OD, 300 MHz): *δ* 7.13 (d, *J* = 8.1 Hz, 1H, CH-16), 7.10 (s, 1H, CH-2), 7.05 (t, *J* = 7.2 Hz, 1H, CH-15), 6.98 (d, *J* = 6.8 Hz, 1H, CH-14), 3.97 (d, *J* = 11.1 Hz, 1H, CH-5), 3.58 (dd, *J* = 10.9, *J* = 6.2 Hz, 1H, CH-4), 3.00 (m, 2H, CH_2-12), 2.50 (m, 1H, CH-11), 2.40 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.60 (s, 3H, CH_3). – **^{13}C NMR** (CD_3OD, 125 MHz): *δ* 135.1 (C_q-17), 130.0 (C_q-13), 127.3 (C_q-18), 123.3 (CH-15), 122.1 (CH-2), 116.7 (CH-14), 110.9 (C_q-3), 109.6 (CH-16), 106.2 (C_q-7), 72.2 (CH-5), 64.0 (C_q-10), 55.0 (CH-11), 37.8 (CH-4), 36.8 (CH_3-20), 27.7 (CH_2-12), 26.4 (CH_3-22), 25.2 (CH_3-21). – (+)-**ESIMS**: *m/z* = 359 $[M+Na]^+$, 695 $[2M+Na]^+$. – (-)-**ESIMS**: *m/z* = 335 $[M-H]^-$, 693 $[2M+Na-2H]^-$. – (+)-**HRESIMS**: *m/z* = 359.1366 $[M+Na]^+$ (359.1366 Calcd. for $C_{20}H_{20}N_2NaO_3$). – (-)-**HRESIMS**: *m/z* = 335.1417 $[M-H]^-$ (335.1401 Calcd. for $C_{20}H_{19}N_2O_3$).

6.18 *Aspergillus oryzae*

6.18.1 Pre-screening

The crude extract showed in the agar diffusion test the antimicrobial activity.

Table 27: The biological activity for *Aspergillus oryzae* sp.

Fungal Extract	Agar diffusion test (40 *μ*g/disc (∅ 5 mm); Diameter of inhibition zones in [mm]					
	E. coli	*PS*	*B. subtilis*	*St. aureus*	*Asp. niger*	*C. albicans*
Supernatant	7	9	9	8	9	-
Mycelia	9	12	10	10	8	-

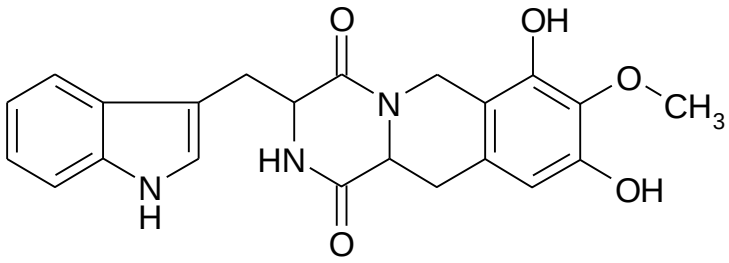
6.18.2 Fermentation and work-up:

The terrestrial *Aspergillus oryzae* sp. was inoculated from well grown agar plates with brownish green air mycelia and light brownish-green colonies into 100 of 1 l-Erlenmeyer flasks, each containing 300 ml of production M_2 medium (g/l): Malt extract (10), peptone (4), glucose (4) and demineralised water (100 %). The pH was adjusted to 7.8 using 2N NaOH before sterilisation. Fermentation was carried out at 180 rpm on a rotary shaker for 10 days at 28 °C. After cultivation, the culture broth was filtered over Celite under pressure. The mycelial extract was macerated in methanol (3 times), and the methanol extract was then concentrated *in vacuo*, and the remaining water residue was re-extracted by ethyl acetate and concentrated, affording 5.1 g as dark green crude extract. The filtrate was extracted with XAD-16, and the adsorbed organic material was extracted with aqueous methanol. The methanol extract was evaporated *in vacuo* and the residual water was extracted with ethyl acetate followed by concentration to afford 11.4 g as dark green crude extract. Both organic extracts were combined, as TLC showed identity.

6.18.3 Scale up and isolation:

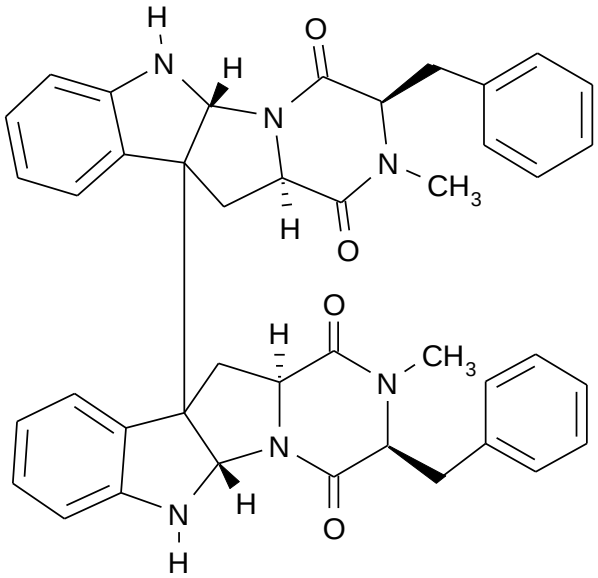
The whole crude extract (16.5 g) was applied to column chromatography on silica gel (40 × 10 cm) and eluted with cyclohexane-CH_2Cl_2-MeOH gradient. According to TLC monitoring, six fractions were obtained; FI (4.57 g), FII (2.1 g), FIII (0.85 g), FIV (1.55 g), FV (1.74 g) and FVI (1.99 g). Purification of fraction II using different Sephadex LH-20 column chromatography afforded colourless solid of ditryptophenaline (**136,** 10 mg). An application of fraction III to Sephadex LH-20 (CH_2Cl_2 /40 % MeOH) delivered two colourless solids of kojic acid (**127**, 100 mg) and α-cyclopiazonic acid (**130**, 25 mg). Purification of Fraction IV on a column of Sephadex LH-20 (MeOH) gave colourless solid of 7,8-dihydroxy-3-(1*H*-indole-3-ylmethyl)-10-methoxy-2,3,11,11a-tetrahydro-6H-pyrazino[1,2-b]isoquinoline-1,4-dione (**134**, 11 mg).

7,9-Dihydroxy-3-(1H-indol-3-ylmethyl)-8-methoxy-2,3,11,11a-tetrahydro-6H-pyrazino[1,2-b]isoquinoline-1,4-dione (**134**)



Colourless solid, exhibiting an UV absorbance and violet changed to blue colouration on spraying with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.39 (CH_2Cl_2/10% MeOH). – $\boldsymbol{[\alpha]}_D^{20}$ (-200° (*c* = 0.12, MeOH). – **^{1}H NMR** (CD_3OD, 300 MHz) and **^{13}C NMR** (CD_3OD, 125 MHz)see Table 17– ^{1}H,^{1}H **COSY** and **HMBC** correlation Figure 229. − **UV/VIS:** λ_{max} (log ε) (MeOH): 218 (4.24), 272 (3.64), 289 sh (3.53); (MeOH/HCl): 219 (4.23), 271 (3.63), 289 sh (3.53); (MeOH/NaOH): 221 (4.26), 281 (3.67), 289 sh (3.62) nm. – (+)-**ESIMS** *m/z* (%) 430 ($[M+Na]^+$, 4), 837 ($[2M+Na]^+$, 4). – (–)-**ESIMS**: *m/z* = 406 $[M-H]^-$, 813 $[2M-H]^-$. – (+)-**HRESIMS**: *m/z* = 430.1361 $[M+Na]^+$ (calcd. 430.1373 for $C_{22}H_{21}NaN_3O_5$). – (-)-**HRESIMS**: *m/z* = 406.1392 $[M-H]^-$ (calcd. 406.1408 for $C_{22}H_{20}N_3O_5$).

Ditryptophenaline (136)



Colourless solid, UV absorbing, changed to blue colouration on spraying with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.35 (CH_2Cl_2/10% MeOH). – **^{1}H NMR** ($CDCl_3$, 300 MHz) and **^{13}C NMR** ($CDCl_3$, 125 MHz) see Table 18. – (+)-**ESIMS** *m/z* 715 $[M+Na]^+$. – (-)-**ESIMS**: *m/z* = 691 $[M-H]^-$ **HRESIMS**: *m/z* = 715.2993 $[M+Na]^+$ (calcd. 715.3003 for $C_{42}H_{40}N_6NaO_4$). − (-)-**HRESIMS**: *m/z* = 691.3043 $[M-H]^-$ (calcd. 691.3038 for $C_{42}H_{39}N_6O_4$).

6.19 Endophytic fungus *Aspergillus fumigatus* R7

6.19.1 Pre-screening

The crude extract of endophytic R7 showed in the agar diffusion test activity against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü57), *Staphylococcus aureus*, and good activity against *Artemia salina.*

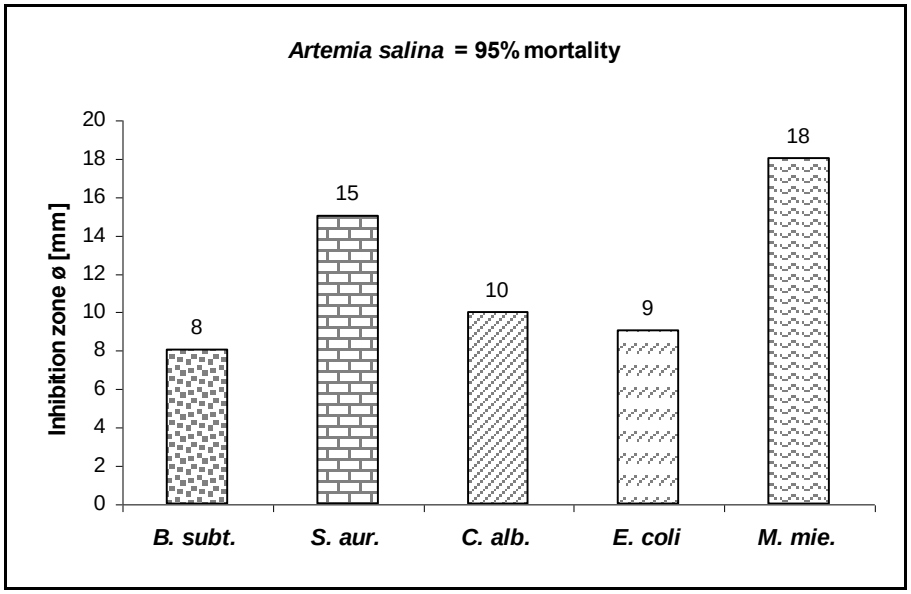


Figure 259: Biological activity of the crude extract of fungus *Aspergillus fumigatus* endophytic R7

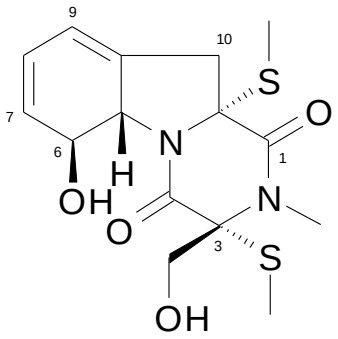
6.19.2 Fermentation and working up

This endophytic R7 formed brown mycelial colonies. A 30-liter shaker culture of the terrestrial streptomycete strain was incubating at 28 °C using M_2^+ agar medium. The resulting green culture broth was harvested after 7 days, mixed with ca. 1 kg diatomaceous earth (Celite) and pressed through a filter press to afford the aqueous filtrate and a mycelial fraction. The aqueous phase was extracted separately using XAD-16 resin followed by elution with MeOH/H_2O. The aqueous methanolic extract was concentrated and the water residue was again extracted with ethyl acetate. The mycelium was extracted with ethyl acetate (3 times) followed by acetone (3 times). The EtOAc and acetone phases were evaporated and dryness. On TLC the three crude extracts showed similar zones, accordingly they were collected together, the extract was defatted with cyclohexane by decantation to get 8.9 g of greenish-brown crude extract.

6.19.3 Scale up and isolation

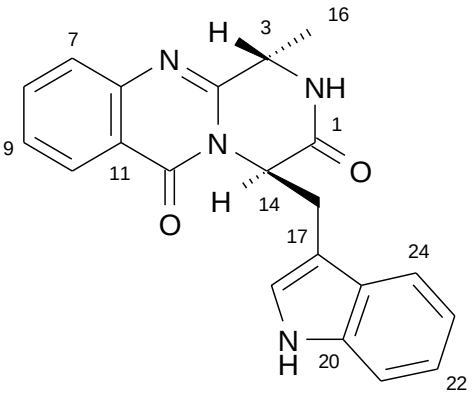
The whole crude extract (8.7 g) was applied to column chromatography on silica gel (40 × 10 cm) and eluted with cyclohexane-CH_2Cl_2-MeOH gradient. According to TLC monitoring, 2 fractions were obtained beside the fatty acid fraction; FII was applied to purified by PTLC chromatogram and then purified again by using Sephadex LH-20 and eluted by MeOH to deliver FR-49175 (**137**)**,** fumiquinzoline-F (**138**) and fumiquinzoline-D (**139**). Fraction III also purified by using PTLC chromatogram and then purified again by using Sephadex LH-20 to afford (Z,Z)-N,N'-[1-[(4-hydroxyphenyl)methylene]-2-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-formamide (**141**) and pyrrolizin-3-one trimer (**144**), see Figure 237.

Bisdethio(bismethylthio)gliotoxin (137):



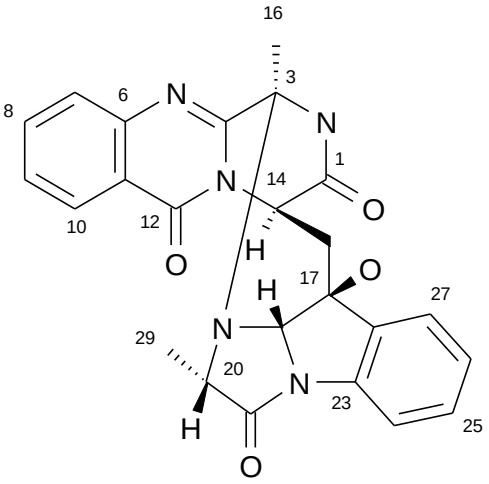
Oliy substance, UV absorbance at 265 nm turned to blue colour with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.25 (CH_2Cl_2/10% MeOH). – **^{13}C** and **^{1}H NMR** (125, 300 MHz, CD_3OD) see Table 19. – (+)-**ESIMS** *m/z* 379 $[M+Na]^+$, 735 $[2M+Na]^+$. – (+)-**HRESIMS** *m/z* 379.0756 (calcd. 379.0757 for $C_{15}H_{20}N_2NaO_4S_2$)

Fumiquinazoline-F (138):



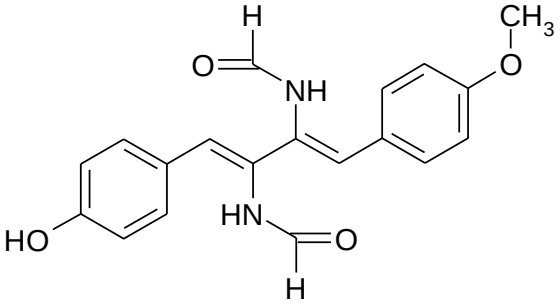
Oily substance, UV active, turned to yellow colour with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.27 (CH_2Cl_2/10% MeOH). – **^{13}C** and **^{1}H NMR** (125, 300 MHz, CD_3OD) see Table 20. – (+)-**ESIMS** *m/z* 381 $[M+Na]^+$, 739 $[2M+Na]^+$, 1097 $[3M+Na]^+$. – (-)-**ESIMS** *m/z* 357 $[M-H]^-$, 715 $[2M-H]^-$. – (+)-**HRESIMS** *m/z* 381.1316 (calcd. 381.1322 for $C_{21}H_{18}N_4NaO_2$). − (-)-**HRESIMS** *m/z* 357.1349 $[M-H]^-$ (calcd. 357.1357 for $C_{21}H_{17}N_4O_2$).

Fumiquinazoline-D (**139**):



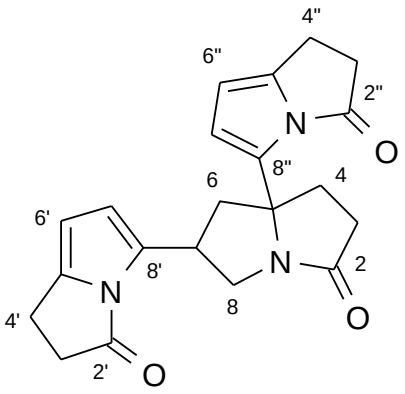
Colourless solid, UV active substance, turned to blue colouration with anisaldehyde reagent and heating – $\boldsymbol{R_f}$ = 0.18 (CH_2Cl_2/5% CH_3OH). – **^{13}C** and **^{1}H NMR** (125, 300 MHz, CD_3OD) see Table 21. – (+)-**ESIMS** *m/z* 466 $[M+Na]^+$, 909 $[2M+Na]^+$, 1352 $[3M+Na]^+$. − (-)-**ESIMS**: *m/z* = 442 $[M-H]^-$, 885 $[2M-H]^-$,− (+)-**HRESIMS**: *m/z* = 466.1471 (calcd. 466.1486 for $C_{24}H_{21}N_5NaO_4$). – (-)-**HRESIMS**: *m/z* = 442.1509 $[M-H]^-$ (calcd. 442.1521 for $C_{24}H_{20}N_5O_4$).

(Z,Z)-N,N'-[1-[(4-Hydroxyphenyl)methylene]-2-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-formamide (141):



Colourless solid, UV absorbance, turned to yellow with anisaldehyde/sulphuric acid. – $\boldsymbol{R_f}$ = 0.20 (CH_2Cl_2/5% CH_3OH) – **^{1}H NMR** (300 MHz, DMSO-d_6) *cis* to *trans* ratio (1.2: 3) 9.64 [s (br), OH], 9.54/9.51/9.43/9.40 [s, NH (*cis*)], 9.37/9.33/9.28/9.24 [d, *J* = 11 Hz, NH (trans)], 8.20/ 8.19/ 8.18 [s, CHO (cis)], 7.86/ 7.79 [d, *J* = 11 Hz, CHO (trans)], 7.5-7.3 (s, H-4/H-4'), 6.9-7.0/6.7-6.8 (s, H-5/H-5'), 6.53/6.52/6.51/6.49/6.46/6.45 (s, H-2/H-2'), 3.77/3.76 (s, CH_3-8'). –**^{13} C NMR** (MeOH, 150 MHz) see Table 22. – (+)-**ESIMS**: *m/z* = 361 $[M+Na]^+$, 699 $[2M+Na]^+$. − (-)-**ESIMS**: *m/z* = 337 $[M-H]^-$, 675 $[2M-H]^-$. – (+)-**HRESIMS**: *m/z* = 361.1151 (calcd. 361.1159 for $C_{19}H_{18}N_2NaO_4$). – (-)-**HRESIMS**: *m/z* = 337.1190 $[M-H]^-$ (calcd. 337.1194 for $C_{19}H_{17}N_2O_4$).

Pyrrolizin-3-one trimer (144):



Colourless oil, UV active, turned to blue with anisaldehyde/sulphuric acid and heating. – $\boldsymbol{R_f}$ = 0.23 (CH_2Cl_2/5% CH_3OH). – **^{1}H NMR** and **^{13}C NMR** (MeOH, 300, 150 MHz) see Table 23. – (-)-**ESIMS**: *m/z* = 337 $[M-H]^-$, 675 $[2M-H]^-$. – (+)-**HRESIMS**: *m/z* = 361.1151 (calcd. 361.1159 for $C_{19}H_{18}N_2NaO_4$). – (-)-**HRESIMS**: *m/z* = 337.1190 $[M-H]^-$ (calcd. 337.1194 for $C_{19}H_{17}N_2O_4$).

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